U.S. Environmental Protection Agency, National Center for Computational Toxicology (U.S. EPA NCCT)

TOXICITY FORECASTER (TOXCAST) *IN VITRO* ASSAYS

Assay Documentation for Non-Guideline *In Vitro* Test Methods

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919-541-4219

ASSAY SOURCES

Endocrine Assays (multiple sources)

ACEA Biosciences, Inc. (ACEA)

Apredica, LLC (APR)

ArunA Biomedical, Inc. (ARUNA)

Attagene, Inc. (ATG, XTT)

BioReliance Corporation (BRL)

BioSeek, Inc. (BSK)

CeeTox, Inc. (CEETOX)

CellzDirect, Inc. (CLD)

HuREL Corporation (HuREL)

Life Technologies Corporation Expression Analysis (LTEA)

National Center for Computational Toxicology, US EPA (NCCT)

National Health and Environmental Effects Research Lab, US EPA (NHEERL, NIS)

NovaScreen Biosciences Corporation (NVS)

Odyssey Thera, Inc. (OT)

Stemina Biomarker Discovery, Inc. (STEMINA)

Tanguay Lab, Oregon State University (TANGUAY)

Toxicity Testing in the 21st Century (TOX21)

VALA Sciences, Inc. (VALA)

ASSAY DOCUMENTATION

Endocrine Assays

1. ACEA_T47D_80hr_Positive
2. ATG_AR_TRANS_up
3. ATG_ERa_TRANS_up
4. ATG_ERb_TRANS2_up
5. ATG_ERE_CIS_up
6. ATG_ERRa_TRANS_up
7. ATG_ERRb_TRANS2_up
8. ATG_ERRg_TRANS_up
9. ATG_FXR_TRANS_up
10. ATG_IR1_CIS_up
11. ATG_PPARa_TRANS_up
12. ATG_PPARd_TRANS_up
13. ATG_PPARg_TRANS_up
14. ATG_PPRE_CIS_up
15. ATG_THRa1_TRANS_up
16. ATG_THRb_TRANS2_up
17. CEETOX_H295R_11DCORT_dn
18. CEETOX_H295R_11DCORT_up
19. CEETOX_H295R_ANDR_dn
20. CEETOX_H295R_ANDR_up
21. CEETOX_H295R_CORTIC_dn
22. CEETOX_H295R_CORTIC_up
23. CEETOX_H295R_CORTISOL_dn
24. CEETOX_H295R_CORTISOL_up
25. CEETOX_H295R_DHEA_dn
26. CEETOX_H295R_DHEA_up
27. CEETOX_H295R_DOC_dn
28. CEETOX_H295R_DOC_up
29. CEETOX_H295R_ESTRADIOL_dn
30. CEETOX_H295R_ESTRADIOL_up
31. CEETOX_H295R_ESTRONE_dn
32. CEETOX_H295R_ESTRONE_up
33. CEETOX_H295R_OHPREG_dn
34. CEETOX_H295R_OHPREG_up
35. CEETOX_H295R_OHPROG_dn
36. CEETOX_H295R_OHPROG_up
37. CEETOX_H295R_PREG_dn
38. CEETOX_H295R_PREG_up
39. CEETOX_H295R_PROG_dn
40. CEETOX_H295R_PROG_up
41. CEETOX_H295R_TESTO_dn
42. CEETOX_H295R_TESTO_up
43. LTEA_HepaRG_THRSP_dn
44. LTEA_HepaRG_THRSP_up
45. NVS_NR_bER
46. NVS_NR_cAR
47. NVS_NR_hAR
48. NVS_NR_hER
49. NVS_NR_hFXR_Agonist
50. NVS_NR_hFXR_Antagonist
51. NVS_NR_hPPARa
52. NVS_NR_hPPARg
53. NVS_NR_hTRa_Antagonist
54. NVS_NR_mERa
55. NVS_NR_rAR
56. OT_AR_ARELUC_AG_1440
57. OT_AR_ARSRC1_0480
58. OT_AR_ARSRC1_0960
59. OT_ER_ERaERa_0480
60. OT_ER_ERaERa_1440
61. OT_ER_ERaERb_0480
62. OT_ER_ERaERb_1440
63. OT_ER_ERbERb_0480
64. OT_ER_ERbERb_1440
65. OT_ERa_EREGFP_0120
66. OT_ERa_EREGFP_0480
67. OT_ERa_ERELUC_AG_1440
68. OT_ERa_ERELUC_ANT_1440
69. OT_ERb_ERELUC_ANT_1440
70. OT_FXR_FXRSRC1_0480
71. OT_FXR_FXRSRC1_1440
72. OT_PPARg_PPARgSRC1_0480
73. OT_PPARg_PPARgSRC1_1440
74. Tox21_AR_BLA_Agonist_ratio
75. Tox21_AR_BLA_Antagonist_ratio
76. Tox21_AR_LUC_MDAKB2_Agonist
77. Tox21_AR_LUC_MDAKB2_Agonist_3uM_Nilutamide
78. Tox21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881
79. Tox21_AR_LUC_MDAKB2_Antagonist_10nM_R1881
80. Tox21_ERa_BLA_Agonist_ratio
81. Tox21_ERa_BLA_Antagonist_ratio
82. Tox21_ERa_LUC_VM7_Agonist
83. Tox21_ERa_LUC_VM7_Agonist_10nM_ICI182780
84. Tox21_ERa_LUC_VM7_Antagonist_0.1nM_E2
85. Tox21_ERa_LUC_VM7_Antagonist_0.5nM_E2
86. Tox21_FXR_BLA_agonist_ratio
87. Tox21_FXR_BLA_antagonist_ratio
88. Tox21_PPARd_BLA_Agonist_ratio
89. Tox21_PPARd_BLA_antagonist_ratio
90. Tox21_PPARg_BLA_Agonist_ratio
91. Tox21_PPARg_BLA_antagonist_ratio
92. Tox21_TR_LUC_GH3_Agonist
93. Tox21_TR_LUC_GH3_Antagonist

ASSAY DOCUMENTATION

Partial assay information available

94. ACEA_AR_agonist_80hr
95. ACEA_AR_agonist_AUC_viability
96. ACEA_AR_antagonist_80hr
97. ACEA_AR_antagonist_AUC_viability
98. ACEA_ER_80hr
99. ACEA_ER_AUC_viability
100. ACEA_HuRELhumanA_AUC_ABT_309h
101. ACEA_HuRELhumanA_AUC_ABT_48h
102. ACEA_HuRELhumanA_AUC_Con_309h
103. ACEA_HuRELhumanA_AUC_Con_48h
104. ACEA_HuRELhumanB_AUC_ABT_309h
105. ACEA_HuRELhumanB_AUC_ABT_48h
106. ACEA_HuRELhumanB_AUC_Con_309h
107. ACEA_HuRELhumanB_AUC_Con_48h
108. ACEA_HuRELhumanC_AUC_ABT_309h
109. ACEA_HuRELhumanC_AUC_ABT_48h
110. ACEA_HuRELhumanC_AUC_Con_309h
111. ACEA_HuRELhumanC_AUC_Con_48h
112. ACEA_HuRELrat_AUC_ABT_309h
113. ACEA_HuRELrat_AUC_ABT_48h
114. ACEA_HuRELrat_AUC_Con_309h
115. ACEA_HuRELrat_AUC_Con_48h
116. ACEA_SERM_Agonist_ECC-1_72hr
117. ACEA_SERM_Agonist_Ishikawa_72hr
118. ACEA_SERM_Agonist_LNCaP_72hr
119. ACEA_SERM_Antagonist_ECC-1_72hr
120. ACEA_SERM_Antagonist_Ishikawa_72hr
121. ACEA_SERM_Antagonist_LNCaP_72hr

122. APR_Cell_count_1_24hr_dn
123. APR_Cell_count_1_24hr_up
124. APR_Cell_count_1_72hr_dn
125. APR_Cell_count_1_72hr_up
126. APR_Cell_count_2_24hr_dn
127. APR_Cell_count_2_24hr_up
128. APR_Cell_count_2_72hr_dn
129. APR_Cell_count_2_72hr_up
130. APR_Cell_cycle_arrest_1_24hr_dn
131. APR_Cell_cycle_arrest_1_24hr_up
132. APR_Cell_cycle_arrest_1_72hr_dn
133. APR_Cell_cycle_arrest_1_72hr_up
134. APR_Cell_cycle_arrest_2_24hr_dn
135. APR_Cell_cycle_arrest_2_24hr_up
136. APR_Cell_cycle_arrest_2_72hr_dn
137. APR_Cell_cycle_arrest_2_72hr_up
138. APR_Hepat_Apoptosis_1hr_dn
139. APR_Hepat_Apoptosis_1hr_up
140. APR_Hepat_Apoptosis_24hr_dn
141. APR_Hepat_Apoptosis_24hr_up
142. APR_Hepat_Apoptosis_48hr_dn
143. APR_Hepat_Apoptosis_48hr_up
144. APR_Hepat_CellLoss_1hr_dn
145. APR_Hepat_CellLoss_1hr_up
146. APR_Hepat_CellLoss_24hr_dn
147. APR_Hepat_CellLoss_24hr_up
148. APR_Hepat_CellLoss_48hr_dn
149. APR_Hepat_CellLoss_48hr_up
150. APR_Hepat_DNADamage_1hr_dn
151. APR_Hepat_DNADamage_1hr_up
152. APR_Hepat_DNADamage_24hr_dn
153. APR_Hepat_DNADamage_24hr_up
154. APR_Hepat_DNADamage_48hr_dn
155. APR_Hepat_DNADamage_48hr_up
156. APR_Hepat_DNATexture_1hr_dn
157. APR_Hepat_DNATexture_1hr_up
158. APR_Hepat_DNATexture_24hr_dn
159. APR_Hepat_DNATexture_24hr_up
160. APR_Hepat_DNATexture_48hr_dn
161. APR_Hepat_DNATexture_48hr_up
162. APR_Hepat_LysosomalMass_1hr_dn
163. APR_Hepat_LysosomalMass_1hr_up
164. APR_Hepat_LysosomalMass_24hr_dn
165. APR_Hepat_LysosomalMass_24hr_up
166. APR_Hepat_LysosomalMass_48hr_dn
167. APR_Hepat_LysosomalMass_48hr_up
168. APR_Hepat_MitoFxnI_1hr_dn
169. APR_Hepat_MitoFxnI_1hr_MEAN_dn
170. APR_Hepat_MitoFxnI_1hr_MEAN_up
171. APR_Hepat_MitoFxnI_1hr_up
172. APR_Hepat_MitoFxnI_24hr_dn
173. APR_Hepat_MitoFxnI_24hr_MEAN_dn
174. APR_Hepat_MitoFxnI_24hr_MEAN_up
175. APR_Hepat_MitoFxnI_24hr_up
176. APR_Hepat_MitoFxnI_48hr_dn
177. APR_Hepat_MitoFxnI_48hr_MEAN_dn
178. APR_Hepat_MitoFxnI_48hr_MEAN_up
179. APR_Hepat_MitoFxnI_48hr_up
180. APR_Hepat_NuclearSize_1hr_dn
181. APR_Hepat_NuclearSize_1hr_up
182. APR_Hepat_NuclearSize_24hr_dn
183. APR_Hepat_NuclearSize_24hr_up
184. APR_Hepat_NuclearSize_48hr_dn
185. APR_Hepat_NuclearSize_48hr_up
186. APR_Hepat_Steatosis_1hr_dn
187. APR_Hepat_Steatosis_1hr_up
188. APR_Hepat_Steatosis_24hr_dn
189. APR_Hepat_Steatosis_24hr_up
190. APR_Hepat_Steatosis_48hr_dn
191. APR_Hepat_Steatosis_48hr_TotalArea_dn
192. APR_Hepat_Steatosis_48hr_TotalArea_up
193. APR_Hepat_Steatosis_48hr_up
194. APR_HepG2_CellCycleArrest_1h_dn
195. APR_HepG2_CellCycleArrest_1h_up
196. APR_HepG2_CellCycleArrest_24h_dn
197. APR_HepG2_CellCycleArrest_24h_up
198. APR_HepG2_CellCycleArrest_72h_dn
199. APR_HepG2_CellCycleArrest_72h_up
200. APR_HepG2_CellLoss_1h_dn
201. APR_HepG2_CellLoss_1h_up
202. APR_HepG2_CellLoss_24h_dn
203. APR_HepG2_CellLoss_24h_up
204. APR_HepG2_CellLoss_72h_dn
205. APR_HepG2_CellLoss_72h_up
206. APR_HepG2_MicrotubuleCSK_1h_dn
207. APR_HepG2_MicrotubuleCSK_1h_up

208. APR_HepG2_MicrotubuleCSK_24h_dn
209. APR_HepG2_MicrotubuleCSK_24h_up
210. APR_HepG2_MicrotubuleCSK_72h_dn
211. APR_HepG2_MicrotubuleCSK_72h_up
212. APR_HepG2_MitoMass_1h_dn
213. APR_HepG2_MitoMass_1h_up
214. APR_HepG2_MitoMass_24h_dn
215. APR_HepG2_MitoMass_24h_up
216. APR_HepG2_MitoMass_72h_dn
217. APR_HepG2_MitoMass_72h_up
218. APR_HepG2_MitoMembPot_1h_dn
219. APR_HepG2_MitoMembPot_1h_up
220. APR_HepG2_MitoMembPot_24h_dn
221. APR_HepG2_MitoMembPot_24h_up
222. APR_HepG2_MitoMembPot_72h_dn
223. APR_HepG2_MitoMembPot_72h_up
224. APR_HepG2_MitoticArrest_1h_dn
225. APR_HepG2_MitoticArrest_1h_up
226. APR_HepG2_MitoticArrest_24h_dn
227. APR_HepG2_MitoticArrest_24h_up
228. APR_HepG2_MitoticArrest_72h_dn
229. APR_HepG2_MitoticArrest_72h_up
230. APR_HepG2_NuclearSize_1h_dn
231. APR_HepG2_NuclearSize_1h_up
232. APR_HepG2_NuclearSize_24h_dn
233. APR_HepG2_NuclearSize_24h_up
234. APR_HepG2_NuclearSize_72h_dn
235. APR_HepG2_NuclearSize_72h_up
236. APR_HepG2_OxidativeStress_1h_dn
237. APR_HepG2_OxidativeStress_1h_up
238. APR_HepG2_OxidativeStress_24h_dn
239. APR_HepG2_OxidativeStress_24h_up
240. APR_HepG2_OxidativeStress_72h_dn
241. APR_HepG2_OxidativeStress_72h_up
242. APR_HepG2_p53Act_1h_dn
243. APR_HepG2_p53Act_1h_up
244. APR_HepG2_p53Act_24h_dn
245. APR_HepG2_p53Act_24h_up
246. APR_HepG2_p53Act_72h_dn
247. APR_HepG2_p53Act_72h_up
248. APR_HepG2_StressKinase_1h_dn
249. APR_HepG2_StressKinase_1h_up
250. APR_HepG2_StressKinase_24h_dn
251. APR_HepG2_StressKinase_24h_up
252. APR_HepG2_StressKinase_72h_dn
253. APR_HepG2_StressKinase_72h_up
254. APR_Mitochondrial_mass_24hr_dn
255. APR_Mitochondrial_mass_24hr_up
256. APR_Mitochondrial_mass_72hr_dn
257. APR_Mitochondrial_mass_72hr_up
258. APR_Mitochondrial_membrane_potential_24hr_dn
259. APR_Mitochondrial_membrane_potential_24hr_up
260. APR_Mitochondrial_membrane_potential_72hr_dn
261. APR_Mitochondrial_membrane_potential_72hr_up
262. APR_Nuclear_size_1_24hr_dn
263. APR_Nuclear_size_1_24hr_up
264. APR_Nuclear_size_1_72hr_dn
265. APR_Nuclear_size_1_72hr_up
266. APR_Nuclear_size_2_24hr_dn
267. APR_Nuclear_size_2_24hr_up
268. APR_Nuclear_size_2_72hr_dn
269. APR_Nuclear_size_2_72hr_up
270. APR_p-cJun_24hr_dn
271. APR_p-cJun_24hr_up
272. APR_p-cJun_72hr_dn
273. APR_p-cJun_72hr_up
274. APR_p-H2AX_24hr_dn
275. APR_p-H2AX_24hr_up
276. APR_p-H2AX_72hr_dn
277. APR_p-H2AX_72hr_up
278. APR_p-H3_24hr_dn
279. APR_p-H3_24hr_up
280. APR_p-H3_72hr_dn
281. APR_p-H3_72hr_up
282. APR_p53_activation_24hr_dn
283. APR_p53_activation_24hr_up
284. APR_p53_activation_72hr_dn
285. APR_p53_activation_72hr_up
286. APR_Tubulin_microtubule_stability_24hr_dn
287. APR_Tubulin_microtubule_stability_24hr_up
288. APR_Tubulin_microtubule_stability_72hr_dn
289. APR_Tubulin_microtubule_stability_72hr_up

290. ARUNA_H9_hNC_CT
291. ARUNA_H9_hNC_MG_dn
292. ARUNA_H9_hNC_MG_up
293. ARUNA_H9_hNN_NO_BranchPtsperNeuron_dn
294. ARUNA_H9_hNN_NO_BranchPtsperNeuron_up
295. ARUNA_H9_hNN_NO_NeuriteCountperNeuron_dn
296. ARUNA_H9_hNN_NO_NeuriteCountperNeuron_up
297. ARUNA_H9_hNN_NO_NeuriteLengthperNeuron_dn
298. ARUNA_H9_hNN_NO_NeuriteLengthperNeuron_up
299. ARUNA_H9_hNN_NO_NucleusCount_dn
300. ARUNA_H9_hNN_NO_NucleusCount_up
301. ARUNA_H9_hNP_CT
302. ARUNA_H9_hNP_MG_dn
303. ARUNA_H9_hNP_MG_up

304. ATG_Ahr_CIS_dn
305. ATG_Ahr_CIS_up
306. ATG_AP_1_CIS_dn
307. ATG_AP_1_CIS_up
308. ATG_AP_2_CIS_dn
309. ATG_AP_2_CIS_up
310. ATG_AR_TRANS_dn
311. ATG_BRE_CIS_dn
312. ATG_BRE_CIS_up
313. ATG_C_EBP_CIS_dn
314. ATG_C_EBP_CIS_up
315. ATG_CAR_TRANS_dn
316. ATG_CAR_TRANS_up
317. ATG_chAR_TRANS_XSP1_dn
318. ATG_chAR_TRANS_XSP1_up
319. ATG_chAR_XSP2_dn
320. ATG_chAR_XSP2_up
321. ATG_chERa_TRANS_XSP1_dn
322. ATG_chERa_TRANS_XSP1_up
323. ATG_chERa_XSP2_dn
324. ATG_chERa_XSP2_up
325. ATG_CMV_CIS_dn
326. ATG_CMV_CIS_up
327. ATG_COUP_TF1_TRANS2_dn
328. ATG_COUP_TF1_TRANS2_up
329. ATG_COUP_TF2_TRANS2_dn
330. ATG_COUP_TF2_TRANS2_up
331. ATG_CRE_CIS_dn
332. ATG_CRE_CIS_up
333. ATG_DAX1_TRANS2_dn
334. ATG_DAX1_TRANS2_up
335. ATG_DR4_LXR_CIS_dn
336. ATG_DR4_LXR_CIS_up
337. ATG_DR5_CIS_dn
338. ATG_DR5_CIS_up
339. ATG_E_Box_CIS_dn
340. ATG_E_Box_CIS_up
341. ATG_E2F_CIS_dn
342. ATG_E2F_CIS_up
343. ATG_EAR2_TRANS2_dn
344. ATG_EAR2_TRANS2_up
345. ATG_EGR_CIS_dn
346. ATG_EGR_CIS_up
347. ATG_ERa_TRANS_dn
348. ATG_ERb_TRANS2_dn
349. ATG_ERE_CIS_dn
350. ATG_ERRa_TRANS_dn
351. ATG_ERRb_TRANS2_dn
352. ATG_ERRg_TRANS_dn
353. ATG_Ets_CIS_dn
354. ATG_Ets_CIS_up
355. ATG_FoxA2_CIS_dn
356. ATG_FoxA2_CIS_up
357. ATG_FoxO_CIS_dn
358. ATG_FoxO_CIS_up
359. ATG_frAR_TRANS_XSP1_dn
360. ATG_frAR_TRANS_XSP1_up
361. ATG_frAR_XSP2_dn
362. ATG_frAR_XSP2_up
363. ATG_frER1_TRANS_XSP1_dn
364. ATG_frER1_TRANS_XSP1_up
365. ATG_frER1_XSP2_dn
366. ATG_frER1_XSP2_up
367. ATG_frER2_TRANS_XSP1_dn
368. ATG_frER2_TRANS_XSP1_up
369. ATG_frER2_XSP2_dn
370. ATG_frER2_XSP2_up
371. ATG_frTRa_TRANS_XSP1_dn
372. ATG_frTRa_TRANS_XSP1_up
373. ATG_frTRa_XSP2_dn
374. ATG_frTRa_XSP2_up
375. ATG_FXR_TRANS_dn
376. ATG_GAL4_TRANS_dn
377. ATG_GAL4_TRANS_up
378. ATG_GAL4_TRANS_XSP1_dn
379. ATG_GAL4_TRANS_XSP1_up
380. ATG_GAL4_XSP2_dn
381. ATG_GAL4_XSP2_up
382. ATG_GATA_CIS_dn
383. ATG_GATA_CIS_up
384. ATG_GCNF_TRANS2_dn
385. ATG_GCNF_TRANS2_up
386. ATG_GLI_CIS_dn

387. ATG_GLI_CIS_up
388. ATG_GPCR_ADORA2A_TRANS_dn
389. ATG_GPCR_ADORA2A_TRANS_up
390. ATG_GPCR_ADORA2B_TRANS_dn
391. ATG_GPCR_ADORA2B_TRANS_up
392. ATG_GPCR_ADRA1A_TRANS_dn
393. ATG_GPCR_ADRA1A_TRANS_up
394. ATG_GPCR_ADRA2B_TRANS_dn
395. ATG_GPCR_ADRA2B_TRANS_up
396. ATG_GPCR_ADRB1_TRANS_dn
397. ATG_GPCR_ADRB1_TRANS_up
398. ATG_GPCR_ADRB2_TRANS_dn
399. ATG_GPCR_ADRB2_TRANS_up
400. ATG_GPCR_ADRB3_TRANS_dn
401. ATG_GPCR_ADRB3_TRANS_up
402. ATG_GPCR_CHRM1_TRANS_dn
403. ATG_GPCR_CHRM1_TRANS_up
404. ATG_GPCR_CHRM3_TRANS_dn
405. ATG_GPCR_CHRM3_TRANS_up
406. ATG_GPCR_DRD1_TRANS_dn
407. ATG_GPCR_DRD1_TRANS_up
408. ATG_GPCR_DRD5_TRANS_dn
409. ATG_GPCR_DRD5_TRANS_up
410. ATG_GPCR_EDNRA_TRANS_dn
411. ATG_GPCR_EDNRA_TRANS_up
412. ATG_GPCR_GCGR_TRANS_dn
413. ATG_GPCR_GCGR_TRANS_up
414. ATG_GPCR_GPBAR1_TRANS_dn
415. ATG_GPCR_GPBAR1_TRANS_up
416. ATG_GPCR_GPR40_TRANS_dn
417. ATG_GPCR_GPR40_TRANS_up
418. ATG_GPCR_GQ_TRANS_dn
419. ATG_GPCR_GQ_TRANS_up
420. ATG_GPCR_GS_TRANS_dn
421. ATG_GPCR_GS_TRANS_up
422. ATG_GPCR_GS1_TRANS_dn
423. ATG_GPCR_GS1_TRANS_up
424. ATG_GPCR_HRH1_TRANS_dn
425. ATG_GPCR_HRH1_TRANS_up
426. ATG_GPCR_HTR2B_TRANS_dn
427. ATG_GPCR_HTR2B_TRANS_up
428. ATG_GPCR_HTR6_TRANS_dn
429. ATG_GPCR_HTR6_TRANS_up
430. ATG_GPCR_HTR7_TRANS_dn
431. ATG_GPCR_HTR7_TRANS_up
432. ATG_GPCR_LPAR4_TRANS_dn
433. ATG_GPCR_LPAR4_TRANS_up
434. ATG_GPCR_M_06_TRANS_dn
435. ATG_GPCR_M_06_TRANS_up
436. ATG_GPCR_M_19_TRANS_dn
437. ATG_GPCR_M_19_TRANS_up
438. ATG_GPCR_M_32_TRANS_dn
439. ATG_GPCR_M_32_TRANS_up
440. ATG_GPCR_M_61_TRANS_dn
441. ATG_GPCR_M_61_TRANS_up
442. ATG_GPCR_MC1R_TRANS_dn
443. ATG_GPCR_MC1R_TRANS_up
444. ATG_GPCR_MC2R_TRANS_dn
445. ATG_GPCR_MC2R_TRANS_up
446. ATG_GPCR_MC3R_TRANS_dn
447. ATG_GPCR_MC3R_TRANS_up
448. ATG_GPCR_MC4R_TRANS_dn
449. ATG_GPCR_MC4R_TRANS_up
450. ATG_GPCR_MC5R_TRANS_dn
451. ATG_GPCR_MC5R_TRANS_up
452. ATG_GPCR_PTGDR_TRANS_dn
453. ATG_GPCR_PTGDR_TRANS_up
454. ATG_GPCR_PTGER2_TRANS_dn
455. ATG_GPCR_PTGER2_TRANS_up
456. ATG_GPCR_PTGIR_TRANS_dn
457. ATG_GPCR_PTGIR_TRANS_up
458. ATG_GR_TRANS_dn
459. ATG_GR_TRANS_up
460. ATG_GRE_CIS_dn
461. ATG_GRE_CIS_up
462. ATG_hAR_TRANS_XSP1_dn
463. ATG_hAR_TRANS_XSP1_up
464. ATG_hAR_XSP2_dn
465. ATG_hAR_XSP2_up
466. ATG_hERa_TRANS_XSP1_dn
467. ATG_hERa_TRANS_XSP1_up
468. ATG_hERa_XSP2_dn
469. ATG_hERa_XSP2_up
470. ATG_hERb_TRANS_XSP1_dn
471. ATG_hERb_TRANS_XSP1_up
472. ATG_hERb_XSP2_dn
473. ATG_hERb_XSP2_up
474. ATG_HIF1a_CIS_dn
475. ATG_HIF1a_CIS_up
476. ATG_HNF4a_TRANS_dn
477. ATG_HNF4a_TRANS_up
478. ATG_HNF4g_TRANS2_dn
479. ATG_HNF4g_TRANS2_up
480. ATG_HNF6_CIS_dn
481. ATG_HNF6_CIS_up
482. ATG_Hpa5_TRANS_dn

483. ATG_Hpa5_TRANS_up
484. ATG_hPPARg_TRANS_XSP1_dn
485. ATG_hPPARg_TRANS_XSP1_up
486. ATG_hPPARg_XSP2_dn
487. ATG_hPPARg_XSP2_up
488. ATG_HSE_CIS_dn
489. ATG_HSE_CIS_up
490. ATG_hTRa_TRANS_XSP1_dn
491. ATG_hTRa_TRANS_XSP1_up
492. ATG_hTRa_XSP2_dn
493. ATG_hTRa_XSP2_up
494. ATG_hTRb_TRANS_XSP1_dn
495. ATG_hTRb_TRANS_XSP1_up
496. ATG_hTRb_XSP2_dn
497. ATG_hTRb_XSP2_up
498. ATG_IR1_CIS_dn
499. ATG_ISRE_CIS_dn
500. ATG_ISRE_CIS_up
501. ATG_LRH1_TRANS2_dn
502. ATG_LRH1_TRANS2_up
503. ATG_LXRa_TRANS_dn
504. ATG_LXRa_TRANS_up
505. ATG_LXRb_TRANS_dn
506. ATG_LXRb_TRANS_up
507. ATG_M_06_CIS_dn
508. ATG_M_06_CIS_up
509. ATG_M_06_TRANS_dn
510. ATG_M_06_TRANS_up
511. ATG_M_19_CIS_dn
512. ATG_M_19_CIS_up
513. ATG_M_19_TRANS_dn
514. ATG_M_19_TRANS_up
515. ATG_M_32_CIS_dn
516. ATG_M_32_CIS_up
517. ATG_M_32_TRANS_dn
518. ATG_M_32_TRANS_up
519. ATG_M_61_CIS_dn
520. ATG_M_61_CIS_up
521. ATG_M_61_TRANS_dn
522. ATG_M_61_TRANS_up
523. ATG_M06_TRANS_XSP1_dn
524. ATG_M06_TRANS_XSP1_up
525. ATG_M06_XSP2_dn
526. ATG_M06_XSP2_up
527. ATG_M19_TRANS_XSP1_dn
528. ATG_M19_TRANS_XSP1_up
529. ATG_M19_XSP2_dn
530. ATG_M19_XSP2_up
531. ATG_M32_TRANS_XSP1_dn
532. ATG_M32_TRANS_XSP1_up
533. ATG_M32_XSP2_dn
534. ATG_M32_XSP2_up
535. ATG_M61_TRANS_XSP1_dn
536. ATG_M61_TRANS_XSP1_up
537. ATG_M61_XSP2_dn
538. ATG_M61_XSP2_up
539. ATG_mPPARg_TRANS_XSP1_dn
540. ATG_mPPARg_TRANS_XSP1_up
541. ATG_mPPARg_XSP2_dn
542. ATG_mPPARg_XSP2_up
543. ATG_mPXR_TRANS_XSP1_dn
544. ATG_mPXR_TRANS_XSP1_up
545. ATG_mPXR_XSP2_dn
546. ATG_mPXR_XSP2_up
547. ATG_MR_TRANS2_dn
548. ATG_MR_TRANS2_up
549. ATG_MRE_CIS_dn
550. ATG_MRE_CIS_up
551. ATG_Myb_CIS_dn
552. ATG_Myb_CIS_up
553. ATG_Myc_CIS_dn
554. ATG_Myc_CIS_up
555. ATG_NF_kB_CIS_dn
556. ATG_NF_kB_CIS_up
557. ATG_NFI_CIS_dn
558. ATG_NFI_CIS_up
559. ATG_NOR1_TRANS2_dn
560. ATG_NOR1_TRANS2_up
561. ATG_NRF1_CIS_dn
562. ATG_NRF1_CIS_up
563. ATG_NRF2_ARE_CIS_dn
564. ATG_NRF2_ARE_CIS_up
565. ATG_NUR77_TRANS2_dn
566. ATG_NUR77_TRANS2_up
567. ATG_NURR1_TRANS_dn
568. ATG_NURR1_TRANS_up
569. ATG_Oct_MLP_CIS_dn
570. ATG_Oct_MLP_CIS_up
571. ATG_p53_CIS_dn
572. ATG_p53_CIS_up
573. ATG_Pax6_CIS_dn
574. ATG_Pax6_CIS_up
575. ATG_PBREM_CIS_dn
576. ATG_PBREM_CIS_up
577. ATG_PNR_TRANS2_dn
578. ATG_PNR_TRANS2_up

579. ATG_PPARa_TRANS_dn
580. ATG_PPARd_TRANS_dn
581. ATG_PPARg_TRANS_dn
582. ATG_PPRE_CIS_dn
583. ATG_PR_TRANS2_dn
584. ATG_PR_TRANS2_up
585. ATG_PXR_TRANS_dn
586. ATG_PXR_TRANS_up
587. ATG_PXRE_CIS_dn
588. ATG_PXRE_CIS_up
589. ATG_RARa_TRANS_dn
590. ATG_RARa_TRANS_up
591. ATG_RARb_TRANS_dn
592. ATG_RARb_TRANS_up
593. ATG_RARg_TRANS_dn
594. ATG_RARg_TRANS_up
595. ATG_Rev_ERB_A_TRANS2_dn
596. ATG_Rev_ERB_A_TRANS2_up
597. ATG_Rev_ERB_B_TRANS2_dn
598. ATG_Rev_ERB_B_TRANS2_up
599. ATG_RORa_TRANS2_dn
600. ATG_RORa_TRANS2_up
601. ATG_RORb_TRANS_dn
602. ATG_RORb_TRANS_up
603. ATG_RORE_CIS_dn
604. ATG_RORE_CIS_up
605. ATG_RORg_TRANS_dn
606. ATG_RORg_TRANS_up
607. ATG_RXRa_TRANS_dn
608. ATG_RXRa_TRANS_up
609. ATG_RXRb_TRANS_dn
610. ATG_RXRb_TRANS_up
611. ATG_RXRg_TRANS2_dn
612. ATG_RXRg_TRANS2_up
613. ATG_SF_1_TRANS2_dn
614. ATG_SF_1_TRANS2_up
615. ATG_SHP_TRANS2_dn
616. ATG_SHP_TRANS2_up
617. ATG_Sox_CIS_dn
618. ATG_Sox_CIS_up
619. ATG_Sp1_CIS_dn
620. ATG_Sp1_CIS_up
621. ATG_SREBP_CIS_dn
622. ATG_SREBP_CIS_up
623. ATG_STAT3_CIS_dn
624. ATG_STAT3_CIS_up
625. ATG_TA_CIS_dn
626. ATG_TA_CIS_up
627. ATG_TAL_CIS_dn
628. ATG_TAL_CIS_up
629. ATG_TCF_b_cat_CIS_dn
630. ATG_TCF_b_cat_CIS_up
631. ATG_TGFb_CIS_dn
632. ATG_TGFb_CIS_up
633. ATG_THRa1_TRANS_dn
634. ATG_THRb_TRANS2_dn
635. ATG_TLX_TRANS2_dn
636. ATG_TLX_TRANS2_up
637. ATG_TR2_TRANS2_dn
638. ATG_TR2_TRANS2_up
639. ATG_TR4_TRANS2_dn
640. ATG_TR4_TRANS2_up
641. ATG_trAR_TRANS_XSP1_dn
642. ATG_trAR_TRANS_XSP1_up
643. ATG_trAR_XSP2_dn
644. ATG_trAR_XSP2_up
645. ATG_trERa_TRANS_XSP1_dn
646. ATG_trERa_TRANS_XSP1_up
647. ATG_trERa_XSP2_dn
648. ATG_trERa_XSP2_up
649. ATG_trTRa_TRANS_XSP1_dn
650. ATG_trTRa_TRANS_XSP1_up
651. ATG_trTRa_XSP2_dn
652. ATG_trTRa_XSP2_up
653. ATG_VDR_TRANS_dn
654. ATG_VDR_TRANS_up
655. ATG_VDRE_CIS_dn
656. ATG_VDRE_CIS_up
657. ATG_Xbp1_CIS_dn
658. ATG_Xbp1_CIS_up
659. ATG_XTT_Cytotoxicity_dn
660. ATG_XTT_Cytotoxicity_TRANS_XSP1_dn
661. ATG_XTT_Cytotoxicity_TRANS_XSP1_up
662. ATG_XTT_Cytotoxicity_up
663. ATG_zfAR_TRANS_XSP1_dn
664. ATG_zfAR_TRANS_XSP1_up
665. ATG_zfAR_XSP2_dn
666. ATG_zfAR_XSP2_up
667. ATG_zfER1_TRANS_XSP1_dn
668. ATG_zfER1_TRANS_XSP1_up
669. ATG_zfER1_XSP2_dn
670. ATG_zfER1_XSP2_up
671. ATG_zfER2a_TRANS_XSP1_dn
672. ATG_zfER2a_TRANS_XSP1_up
673. ATG_zfER2a_XSP2_dn
674. ATG_zfER2a_XSP2_up

675. ATG_zfER2b_TRANS_XSP1_dn
676. ATG_zfER2b_TRANS_XSP1_up
677. ATG_zfER2b_XSP2_dn
678. ATG_zfER2b_XSP2_up
679. ATG_zfPPARg_TRANS_XSP1_dn
680. ATG_zfPPARg_TRANS_XSP1_up
681. ATG_zfPPARg_XSP2_dn
682. ATG_zfPPARg_XSP2_up
683. ATG_zfTRa_TRANS_XSP1_dn
684. ATG_zfTRa_TRANS_XSP1_up
685. ATG_zfTRa_XSP2_dn
686. ATG_zfTRa_XSP2_up
687. ATG_zfTRb_TRANS_XSP1_dn
688. ATG_zfTRb_TRANS_XSP1_up
689. ATG_zfTRb_XSP2_dn
690. ATG_zfTRb_XSP2_up
691. XTT_Cytotoxicity_XSP2_dn
692. XTT_Cytotoxicity_XSP2_up

693. BRL_CHO_hypodiploid_freq_up
694. BRL_CHO_micronucleus_freq_up
695. BRL_CHO_relative_survival_dn
696. BRL_CHO_S9_hypodiploid_freq_up
697. BRL_CHO_S9_micronucleus_freq_up
698. BRL_CHO_S9_relative_survival_dn

699. BSK_3C_Eselectin_down
700. BSK_3C_Eselectin_up
701. BSK_3C_HLADR_down
702. BSK_3C_HLADR_up
703. BSK_3C_ICAM1_down
704. BSK_3C_ICAM1_up
705. BSK_3C_IL8_down
706. BSK_3C_IL8_up
707. BSK_3C_MCP1_down
708. BSK_3C_MCP1_up
709. BSK_3C_MIG_down
710. BSK_3C_MIG_up
711. BSK_3C_Proliferation_down
712. BSK_3C_Proliferation_up
713. BSK_3C_SRB_down
714. BSK_3C_SRB_up
715. BSK_3C_Thrombomodulin_down
716. BSK_3C_Thrombomodulin_up
717. BSK_3C_TissueFactor_down
718. BSK_3C_TissueFactor_up
719. BSK_3C_uPAR_down
720. BSK_3C_uPAR_up
721. BSK_3C_VCAM1_down
722. BSK_3C_VCAM1_up
723. BSK_3C_Vis_down
724. BSK_3C_Vis_up
725. BSK_4H_Eotaxin3_down
726. BSK_4H_Eotaxin3_up
727. BSK_4H_MCP1_down
728. BSK_4H_MCP1_up
729. BSK_4H_Pselectin_down
730. BSK_4H_Pselectin_up
731. BSK_4H_SRB_down
732. BSK_4H_SRB_up
733. BSK_4H_uPAR_down
734. BSK_4H_uPAR_up
735. BSK_4H_VCAM1_down
736. BSK_4H_VCAM1_up
737. BSK_4H_VEGFRII_down
738. BSK_4H_VEGFRII_up
739. BSK_BE3C_HLADR_down
740. BSK_BE3C_HLADR_up
741. BSK_BE3C_IL1a_down
742. BSK_BE3C_IL1a_up
743. BSK_BE3C_IP10_down
744. BSK_BE3C_IP10_up
745. BSK_BE3C_MIG_down
746. BSK_BE3C_MIG_up
747. BSK_BE3C_MMP1_down
748. BSK_BE3C_MMP1_up
749. BSK_BE3C_PAI1_down
750. BSK_BE3C_PAI1_up
751. BSK_BE3C_SRB_down
752. BSK_BE3C_SRB_up
753. BSK_BE3C_TGFb1_down
754. BSK_BE3C_TGFb1_up
755. BSK_BE3C_tPA_down
756. BSK_BE3C_tPA_up
757. BSK_BE3C_uPA_down
758. BSK_BE3C_uPA_up
759. BSK_BE3C_uPAR_down
760. BSK_BE3C_uPAR_up
761. BSK_CASM3C_HLADR_down
762. BSK_CASM3C_HLADR_up
763. BSK_CASM3C_IL6_down
764. BSK_CASM3C_IL6_up
765. BSK_CASM3C_IL8_down
766. BSK_CASM3C_IL8_up
767. BSK_CASM3C_LDLR_down
768. BSK_CASM3C_LDLR_up
769. BSK_CASM3C_MCP1_down
770. BSK_CASM3C_MCP1_up
771. BSK_CASM3C_MCSF_down
772. BSK_CASM3C_MCSF_up
773. BSK_CASM3C_MIG_down
774. BSK_CASM3C_MIG_up
775. BSK_CASM3C_Proliferation_down
776. BSK_CASM3C_Proliferation_up
777. BSK_CASM3C_SAA_down
778. BSK_CASM3C_SAA_up
779. BSK_CASM3C_SRB_down
780. BSK_CASM3C_SRB_up
781. BSK_CASM3C_Thrombomodulin_down
782. BSK_CASM3C_Thrombomodulin_up
783. BSK_CASM3C_TissueFactor_down

784. BSK_CASM3C_TissueFactor_up
785. BSK_CASM3C_uPAR_down
786. BSK_CASM3C_uPAR_up
787. BSK_CASM3C_VCAM1_down
788. BSK_CASM3C_VCAM1_up
789. BSK_hDFCGF_CollagenIII_down
790. BSK_hDFCGF_CollagenIII_up
791. BSK_hDFCGF_EGFR_down
792. BSK_hDFCGF_EGFR_up
793. BSK_hDFCGF_IL8_down
794. BSK_hDFCGF_IL8_up
795. BSK_hDFCGF_IP10_down
796. BSK_hDFCGF_IP10_up
797. BSK_hDFCGF_MCSF_down
798. BSK_hDFCGF_MCSF_up
799. BSK_hDFCGF_MIG_down
800. BSK_hDFCGF_MIG_up
801. BSK_hDFCGF_MMP1_down
802. BSK_hDFCGF_MMP1_up
803. BSK_hDFCGF_PAI1_down
804. BSK_hDFCGF_PAI1_up
805. BSK_hDFCGF_Proliferation_down
806. BSK_hDFCGF_Proliferation_up
807. BSK_hDFCGF_SRB_down
808. BSK_hDFCGF_SRB_up
809. BSK_hDFCGF_TIMP1_down
810. BSK_hDFCGF_TIMP1_up
811. BSK_hDFCGF_VCAM1_down
812. BSK_hDFCGF_VCAM1_up
813. BSK_KF3CT_ICAM1_down
814. BSK_KF3CT_ICAM1_up
815. BSK_KF3CT_IL1a_down
816. BSK_KF3CT_IL1a_up
817. BSK_KF3CT_IP10_down
818. BSK_KF3CT_IP10_up
819. BSK_KF3CT_MCP1_down
820. BSK_KF3CT_MCP1_up
821. BSK_KF3CT_MMP9_down
822. BSK_KF3CT_MMP9_up
823. BSK_KF3CT_SRB_down
824. BSK_KF3CT_SRB_up
825. BSK_KF3CT_TGFb1_down
826. BSK_KF3CT_TGFb1_up
827. BSK_KF3CT_TIMP2_down
828. BSK_KF3CT_TIMP2_up
829. BSK_KF3CT_uPA_down
830. BSK_KF3CT_uPA_up
831. BSK_LPS_CD40_down
832. BSK_LPS_CD40_up
833. BSK_LPS_Eselectin_down
834. BSK_LPS_Eselectin_up
835. BSK_LPS_IL1a_down
836. BSK_LPS_IL1a_up
837. BSK_LPS_IL8_down
838. BSK_LPS_IL8_up
839. BSK_LPS_MCP1_down
840. BSK_LPS_MCP1_up
841. BSK_LPS_MCSF_down
842. BSK_LPS_MCSF_up
843. BSK_LPS_PGE2_down
844. BSK_LPS_PGE2_up
845. BSK_LPS_SRB_down
846. BSK_LPS_SRB_up
847. BSK_LPS_TissueFactor_down
848. BSK_LPS_TissueFactor_up
849. BSK_LPS_TNFa_down
850. BSK_LPS_TNFa_up
851. BSK_LPS_VCAM1_down
852. BSK_LPS_VCAM1_up
853. BSK_SAg_CD38_down
854. BSK_SAg_CD38_up
855. BSK_SAg_CD40_down
856. BSK_SAg_CD40_up
857. BSK_SAg_CD69_down
858. BSK_SAg_CD69_up
859. BSK_SAg_Eselectin_down
860. BSK_SAg_Eselectin_up
861. BSK_SAg_IL8_down
862. BSK_SAg_IL8_up
863. BSK_SAg_MCP1_down
864. BSK_SAg_MCP1_up
865. BSK_SAg_MIG_down
866. BSK_SAg_MIG_up
867. BSK_SAg_PBMCCytotoxicity_down
868. BSK_SAg_PBMCCytotoxicity_up
869. BSK_SAg_Proliferation_down
870. BSK_SAg_Proliferation_up
871. BSK_SAg_SRB_down
872. BSK_SAg_SRB_up

CEETOX, INC.

873. CEETOX_H295R_11DCORT_noMTC_dn
874. CEETOX_H295R_11DCORT_noMTC_up
875. CEETOX_H295R_ANDR_noMTC_dn
876. CEETOX_H295R_ANDR_noMTC_up
877. CEETOX_H295R_CORTIC_noMTC_dn
878. CEETOX_H295R_CORTIC_noMTC_up
879. CEETOX_H295R_CORTISOL_noMTC_dn
880. CEETOX_H295R_CORTISOL_noMTC_up
881. CEETOX_H295R_DHEA_noMTC_dn
882. CEETOX_H295R_DHEA_noMTC_up
883. CEETOX_H295R_DOC_noMTC_dn
884. CEETOX_H295R_DOC_noMTC_up
885. CEETOX_H295R_ESTRADIOL_noMTC_dn
886. CEETOX_H295R_ESTRADIOL_noMTC_up
887. CEETOX_H295R_ESTRONE_noMTC_dn
888. CEETOX_H295R_ESTRONE_noMTC_up
889. CEETOX_H295R_MTT_Cytotoxicity_dn
890. CEETOX_H295R_MTT_Cytotoxicity_up
891. CEETOX_H295R_OHPREG_noMTC_dn
892. CEETOX_H295R_OHPREG_noMTC_up
893. CEETOX_H295R_OHPROG_noMTC_dn
894. CEETOX_H295R_OHPROG_noMTC_up
895. CEETOX_H295R_PREG_noMTC_dn
896. CEETOX_H295R_PREG_noMTC_up
897. CEETOX_H295R_PROG_noMTC_dn
898. CEETOX_H295R_PROG_noMTC_up
899. CEETOX_H295R_TESTO_noMTC_dn
900. CEETOX_H295R_TESTO_noMTC_up

901. CLD_ABCB1_0hr_negctrl
902. CLD_ABCB1_24hr
903. CLD_ABCB1_48hr
904. CLD_ABCB1_6hr
905. CLD_ABCB11_0hr_negctrl
906. CLD_ABCB11_24hr
907. CLD_ABCB11_48hr
908. CLD_ABCB11_6hr
909. CLD_ABCG2_0hr_negctrl
910. CLD_ABCG2_24hr
911. CLD_ABCG2_48hr
912. CLD_ABCG2_6hr
913. CLD_ACTIN_0hr_negctrl
914. CLD_ACTIN_24hr
915. CLD_ACTIN_48hr
916. CLD_ACTIN_6hr
917. CLD_CYP1A1_0hr_negctrl
918. CLD_CYP1A1_24hr
919. CLD_CYP1A1_48hr
920. CLD_CYP1A1_6hr
921. CLD_CYP1A2_0hr_negctrl
922. CLD_CYP1A2_24hr
923. CLD_CYP1A2_48hr
924. CLD_CYP1A2_6hr
925. CLD_CYP2B6_0hr_negctrl
926. CLD_CYP2B6_24hr
927. CLD_CYP2B6_48hr
928. CLD_CYP2B6_6hr
929. CLD_CYP2C19_0hr_negctrl
930. CLD_CYP2C19_24hr
931. CLD_CYP2C19_48hr
932. CLD_CYP2C19_6hr
933. CLD_CYP2C9_0hr_negctrl
934. CLD_CYP2C9_24hr
935. CLD_CYP2C9_48hr
936. CLD_CYP2C9_6hr
937. CLD_CYP3A4_0hr_negctrl
938. CLD_CYP3A4_24hr
939. CLD_CYP3A4_48hr
940. CLD_CYP3A4_6hr
941. CLD_GAPDH_0hr_negctrl
942. CLD_GAPDH_24hr
943. CLD_GAPDH_48hr
944. CLD_GAPDH_6hr
945. CLD_GSTA2_0hr_negctrl
946. CLD_GSTA2_24hr
947. CLD_GSTA2_48hr
948. CLD_GSTA2_6hr
949. CLD_HMGCS2_0hr_negctrl
950. CLD_HMGCS2_24hr
951. CLD_HMGCS2_48hr
952. CLD_HMGCS2_6hr
953. CLD_SLCO1B1_0hr_negctrl
954. CLD_SLCO1B1_24hr
955. CLD_SLCO1B1_48hr
956. CLD_SLCO1B1_6hr
957. CLD_SULT2A_0hr_negctrl
958. CLD_SULT2A_24hr
959. CLD_SULT2A_48hr
960. CLD_SULT2A_6hr
961. CLD_UGT1A1_0hr_negctrl
962. CLD_UGT1A1_24hr
963. CLD_UGT1A1_48hr
964. CLD_UGT1A1_6hr

965. HuRELmouseFibroblast_AUC_Con_309h
966. HuRELmouseFibroblast_AUC_Con_48h

LIFE TECHNOLOGIES CORPORATION EXPRESSION ANALYSIS

967. LTEA_HepaRG_ABCB1_dn
968. LTEA_HepaRG_ABCB1_up
969. LTEA_HepaRG_ABCB11_dn
970. LTEA_HepaRG_ABCB11_up
971. LTEA_HepaRG_ABCC2_dn
972. LTEA_HepaRG_ABCC2_up
973. LTEA_HepaRG_ABCC3_dn
974. LTEA_HepaRG_ABCC3_up
975. LTEA_HepaRG_ABCG2_dn
976. LTEA_HepaRG_ABCG2_up
977. LTEA_HepaRG_ACLY_dn
978. LTEA_HepaRG_ACLY_up
979. LTEA_HepaRG_ACOX1_dn
980. LTEA_HepaRG_ACOX1_up
981. LTEA_HepaRG_ADK_dn
982. LTEA_HepaRG_ADK_up
983. LTEA_HepaRG_AFP_dn
984. LTEA_HepaRG_AFP_up
985. LTEA_HepaRG_ALPP_dn
986. LTEA_HepaRG_ALPP_up
987. LTEA_HepaRG_APOA5_dn
988. LTEA_HepaRG_APOA5_up
989. LTEA_HepaRG_BAD_dn
990. LTEA_HepaRG_BAD_up
991. LTEA_HepaRG_BAX_dn
992. LTEA_HepaRG_BAX_up
993. LTEA_HepaRG_BCL2_dn
994. LTEA_HepaRG_BCL2_up
995. LTEA_HepaRG_BCL2L11_dn
996. LTEA_HepaRG_BCL2L11_up
997. LTEA_HepaRG_BID_dn
998. LTEA_HepaRG_BID_up
999. LTEA_HepaRG_CASP3_dn
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1001. LTEA_HepaRG_CASP8_dn
1002. LTEA_HepaRG_CASP8_up
1003. LTEA_HepaRG_CAT_dn
1004. LTEA_HepaRG_CAT_up
1005. LTEA_HepaRG_CCND1_dn
1006. LTEA_HepaRG_CCND1_up
1007. LTEA_HepaRG_CCND2_dn
1008. LTEA_HepaRG_CCND2_up
1009. LTEA_HepaRG_CDKN1A_dn
1010. LTEA_HepaRG_CDKN1A_up
1011. LTEA_HepaRG_CFLAR_dn
1012. LTEA_HepaRG_CFLAR_up
1013. LTEA_HepaRG_CYP1A1_dn
1014. LTEA_HepaRG_CYP1A1_up
1015. LTEA_HepaRG_CYP1A2_dn
1016. LTEA_HepaRG_CYP1A2_up
1017. LTEA_HepaRG_CYP24A1_1_dn
1018. LTEA_HepaRG_CYP24A1_1_up
1019. LTEA_HepaRG_CYP24A1_dn
1020. LTEA_HepaRG_CYP24A1_up
1021. LTEA_HepaRG_CYP2B6_dn
1022. LTEA_HepaRG_CYP2B6_up
1023. LTEA_HepaRG_CYP2C19_dn
1024. LTEA_HepaRG_CYP2C19_up
1025. LTEA_HepaRG_CYP2C8_dn
1026. LTEA_HepaRG_CYP2C8_up
1027. LTEA_HepaRG_CYP2C9_dn
1028. LTEA_HepaRG_CYP2C9_up
1029. LTEA_HepaRG_CYP2E1_dn
1030. LTEA_HepaRG_CYP2E1_up
1031. LTEA_HepaRG_CYP3A4_dn
1032. LTEA_HepaRG_CYP3A4_up
1033. LTEA_HepaRG_CYP3A5_dn
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1037. LTEA_HepaRG_CYP4A11_dn
1038. LTEA_HepaRG_CYP4A11_up
1039. LTEA_HepaRG_CYP4A22_dn
1040. LTEA_HepaRG_CYP4A22_up
1041. LTEA_HepaRG_CYP7A1_dn
1042. LTEA_HepaRG_CYP7A1_up
1043. LTEA_HepaRG_DDIT3_dn
1044. LTEA_HepaRG_DDIT3_up
1045. LTEA_HepaRG_EGF_dn
1046. LTEA_HepaRG_EGF_up

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1056. LTEA_HepaRG_FASN_up
1057. LTEA_HepaRG_FMO3_dn
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1064. LTEA_HepaRG_GADD45A_up
1065. LTEA_HepaRG_GADD45B_dn
1066. LTEA_HepaRG_GADD45B_up
1067. LTEA_HepaRG_GADD45G_dn
1068. LTEA_HepaRG_GADD45G_up
1069. LTEA_HepaRG_GCLC_dn
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1073. LTEA_HepaRG_GE_ACTB_dn
1074. LTEA_HepaRG_GE_ACTB_up
1075. LTEA_HepaRG_GE_GAPDH_dn
1076. LTEA_HepaRG_GE_GAPDH_up
1077. LTEA_HepaRG_GE_POLR2A_dn
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1079. LTEA_HepaRG_GSTA2_dn
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1081. LTEA_HepaRG_GSTM3_dn
1082. LTEA_HepaRG_GSTM3_up
1083. LTEA_HepaRG_HGF_dn
1084. LTEA_HepaRG_HGF_up
1085. LTEA_HepaRG_HIF1A_dn
1086. LTEA_HepaRG_HIF1A_up
1087. LTEA_HepaRG_HMGCS2_dn
1088. LTEA_HepaRG_HMGCS2_up
1089. LTEA_HepaRG_HSPA1A_dn
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1091. LTEA_HepaRG_ICAM1_dn
1092. LTEA_HepaRG_ICAM1_up
1093. LTEA_HepaRG_IGF1_dn
1094. LTEA_HepaRG_IGF1_up
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1096. LTEA_HepaRG_IGFBP1_up
1097. LTEA_HepaRG_IL6_dn
1098. LTEA_HepaRG_IL6_up
1099. LTEA_HepaRG_IL6R_dn
1100. LTEA_HepaRG_IL6R_up
1101. LTEA_HepaRG_JUN_dn
1102. LTEA_HepaRG_JUN_up
1103. LTEA_HepaRG_KCNK1_dn
1104. LTEA_HepaRG_KCNK1_up
1105. LTEA_HepaRG_KLK3_dn
1106. LTEA_HepaRG_KLK3_up
1107. LTEA_HepaRG_KRT19_dn
1108. LTEA_HepaRG_KRT19_up
1109. LTEA_HepaRG_LDH_cytotoxicity
1110. LTEA_HepaRG_LIPC_dn
1111. LTEA_HepaRG_LIPC_up
1112. LTEA_HepaRG_LPL_dn
1113. LTEA_HepaRG_LPL_up
1114. LTEA_HepaRG_MIR122_dn
1115. LTEA_HepaRG_MIR122_up
1116. LTEA_HepaRG_MMP1_dn
1117. LTEA_HepaRG_MMP1_up
1118. LTEA_HepaRG_MMP10_dn
1119. LTEA_HepaRG_MMP10_up
1120. LTEA_HepaRG_MMP3_dn
1121. LTEA_HepaRG_MMP3_up
1122. LTEA_HepaRG_MYC_dn
1123. LTEA_HepaRG_MYC_up
1124. LTEA_HepaRG_NFE2L2_dn
1125. LTEA_HepaRG_NFE2L2_up
1126. LTEA_HepaRG_NFKB1_dn
1127. LTEA_HepaRG_NFKB1_up
1128. LTEA_HepaRG_NQO1_dn
1129. LTEA_HepaRG_NQO1_up
1130. LTEA_HepaRG_PDK4_dn
1131. LTEA_HepaRG_PDK4_up
1132. LTEA_HepaRG_PEG10_dn
1133. LTEA_HepaRG_PEG10_up
1134. LTEA_HepaRG_PPP2R4_dn
1135. LTEA_HepaRG_PPP2R4_up
1136. LTEA_HepaRG_PTEN_dn
1137. LTEA_HepaRG_PTEN_up
1138. LTEA_HepaRG_SDHB_dn
1139. LTEA_HepaRG_SDHB_up
1140. LTEA_HepaRG_SLC10A1_dn
1141. LTEA_HepaRG_SLC10A1_up
1142. LTEA_HepaRG_SLC22A1_dn

1143. LTEA_HepaRG_SLC22A1_up
1144. LTEA_HepaRG_SLC22A6_dn
1145. LTEA_HepaRG_SLC22A6_up
1146. LTEA_HepaRG_SLCO1B1_dn
1147. LTEA_HepaRG_SLCO1B1_up
1148. LTEA_HepaRG_STAT3_dn
1149. LTEA_HepaRG_STAT3_up
1150. LTEA_HepaRG_SULT2A1_dn
1151. LTEA_HepaRG_SULT2A1_up
1152. LTEA_HepaRG_TGFA_dn
1153. LTEA_HepaRG_TGFA_up
1154. LTEA_HepaRG_TGFB1_dn
1155. LTEA_HepaRG_TGFB1_up
1156. LTEA_HepaRG_TIMP1_dn
1157. LTEA_HepaRG_TIMP1_up
1158. LTEA_HepaRG_TNFRSF1A_dn
1159. LTEA_HepaRG_TNFRSF1A_up
1160. LTEA_HepaRG_TP53_dn
1161. LTEA_HepaRG_TP53_up
1162. LTEA_HepaRG_UGT1A1_dn
1163. LTEA_HepaRG_UGT1A1_up
1164. LTEA_HepaRG_UGT1A6_dn
1165. LTEA_HepaRG_UGT1A6_up
1166. LTEA_HepaRG_XBP1_dn
1167. LTEA_HepaRG_XBP1_up

1168. NCCT_HEK293T_CellTiterGLO
1169. NCCT_HTTr_Apoptosis_DMEM_12hr
1170. NCCT_HTTr_Apoptosis_DMEM_24hr
1171. NCCT_HTTr_Apoptosis_DMEM_6hr
1172. NCCT_HTTr_Apoptosis_PRF_DMEM_12hr
1173. NCCT_HTTr_Apoptosis_PRF_DMEM_24hr
1174. NCCT_HTTr_Apoptosis_PRF_DMEM_6hr
1175. NCCT_HTTr_Viability_DMEM_12hr
1176. NCCT_HTTr_Viability_DMEM_24hr
1177. NCCT_HTTr_Viability_DMEM_6hr
1178. NCCT_HTTr_Viability_PRF_DMEM_12hr
1179. NCCT_HTTr_Viability_PRF_DMEM_24hr
1180. NCCT_HTTr_Viability_PRF_DMEM_6hr
1181. NCCT_MITO_basal_resp_rate_OCR_dn
1182. NCCT_MITO_basal_resp_rate_OCR_up
1183. NCCT_MITO_inhib_resp_rate_OCR_dn
1184. NCCT_MITO_inhib_resp_rate_OCR_up
1185. NCCT_MITO_max_resp_rate_OCR_dn
1186. NCCT_MITO_max_resp_rate_OCR_up
1187. NCCT_MITO_viability
1188. NCCT_QuantiLum_inhib_2_dn
1189. NCCT_QuantiLum_inhib_dn
1190. NCCT_TPO_AUR_dn
1191. NCCT_TPO_GUA_dn

NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY (US EPA)

1192. NHEERL_GSC_CellCount_dn
1193. NHEERL_GSC_CellCount_up
1194. NHEERL_GSC_GSCvCellCount_dn
1195. NHEERL_GSC_GSCvCellCount_up
1196. NHEERL_MEA_AB
1197. NHEERL_MEA_LDH
1198. NHEERL_MEA_MFR_dn
1199. NHEERL_MEA_MFR_up
1200. NHEERL_MHC_CellCount_dn
1201. NHEERL_MHC_CellCount_up
1202. NHEERL_MHC_MHCvCellCount_dn
1203. NHEERL_MHC_MHCvCellCount_up
1204. NHEERL_ZF_144hpf_TERATOSCORE_up
1205. NIS_HEK293T_CTG_Cytotoxicity
1206. NIS_RAIU_inhibition

1207. NVS_ADME_hCYP19A1
1208. NVS_ADME_hCYP19A1_Activator
1209. NVS_ADME_hCYP1A1
1210. NVS_ADME_hCYP1A1_Activator
1211. NVS_ADME_hCYP1A2
1212. NVS_ADME_hCYP1A2_Activator
1213. NVS_ADME_hCYP1B1
1214. NVS_ADME_hCYP1B1_Activator
1215. NVS_ADME_hCYP2A6
1216. NVS_ADME_hCYP2A6_Activator
1217. NVS_ADME_hCYP2B6
1218. NVS_ADME_hCYP2B6_Activator
1219. NVS_ADME_hCYP2C18
1220. NVS_ADME_hCYP2C18_Activator
1221. NVS_ADME_hCYP2C19
1222. NVS_ADME_hCYP2C19_Activator
1223. NVS_ADME_hCYP2C8
1224. NVS_ADME_hCYP2C8_Activator
1225. NVS_ADME_hCYP2C9
1226. NVS_ADME_hCYP2C9_Activator
1227. NVS_ADME_hCYP2D6
1228. NVS_ADME_hCYP2D6_Activator
1229. NVS_ADME_hCYP2E1
1230. NVS_ADME_hCYP2E1_Activator
1231. NVS_ADME_hCYP2J2
1232. NVS_ADME_hCYP2J2_Activator
1233. NVS_ADME_hCYP3A4
1234. NVS_ADME_hCYP3A4_Activator
1235. NVS_ADME_hCYP3A5
1236. NVS_ADME_hCYP3A5_Activator
1237. NVS_ADME_hCYP4F12
1238. NVS_ADME_hCYP4F12_Activator
1239. NVS_ADME_rCYP1A1
1240. NVS_ADME_rCYP1A1_Activator
1241. NVS_ADME_rCYP1A2
1242. NVS_ADME_rCYP1A2_Activator
1243. NVS_ADME_rCYP2A1
1244. NVS_ADME_rCYP2A1_Activator
1245. NVS_ADME_rCYP2A2
1246. NVS_ADME_rCYP2A2_Activator
1247. NVS_ADME_rCYP2B1
1248. NVS_ADME_rCYP2B1_Activator
1249. NVS_ADME_rCYP2C11
1250. NVS_ADME_rCYP2C11_Activator
1251. NVS_ADME_rCYP2C12
1252. NVS_ADME_rCYP2C12_Activator
1253. NVS_ADME_rCYP2C13
1254. NVS_ADME_rCYP2C13_Activator
1255. NVS_ADME_rCYP2C6
1256. NVS_ADME_rCYP2C6_Activator
1257. NVS_ADME_rCYP2D1
1258. NVS_ADME_rCYP2D1_Activator
1259. NVS_ADME_rCYP2D2
1260. NVS_ADME_rCYP2D2_Activator
1261. NVS_ADME_rCYP2E1
1262. NVS_ADME_rCYP2E1_Activator
1263. NVS_ADME_rCYP3A1
1264. NVS_ADME_rCYP3A1_Activator
1265. NVS_ADME_rCYP3A2
1266. NVS_ADME_rCYP3A2_Activator
1267. NVS_ENZ_hAbl
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1301. NVS_ENZ_hCDK2
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1304. NVS_ENZ_hCDK6_Activator
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1311. NVS_ENZ_hCK2a2b2
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1324. NVS_ENZ_hEphA1_Activator
1325. NVS_ENZ_hEphA2
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1336. NVS_ENZ_hFGFR3_Activator
1337. NVS_ENZ_hFyn
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1346. NVS_ENZ_hIGF1R_Activator
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1348. NVS_ENZ_hIKKa_Activator
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1360. NVS_ENZ_hLynA_Activator
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1362. NVS_ENZ_hLynB_Activator
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1364. NVS_ENZ_hMAPK1_Activator
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1366. NVS_ENZ_hMAPK11_Activator
1367. NVS_ENZ_hMAPK3
1368. NVS_ENZ_hMAPK3_Activator
1369. NVS_ENZ_hMAPKAPK2
1370. NVS_ENZ_hMAPKAPK2_Activator
1371. NVS_ENZ_hMAPKAPK5
1372. NVS_ENZ_hMAPKAPK5_Activator
1373. NVS_ENZ_hMARK1
1374. NVS_ENZ_hMARK1_Activator
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1376. NVS_ENZ_hMet_Activator
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1378. NVS_ENZ_hMMP1_Activator
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1384. NVS_ENZ_hMMP3_Activator
1385. NVS_ENZ_hMMP7
1386. NVS_ENZ_hMMP7_Activator

1387. NVS_ENZ_hMMP9
1388. NVS_ENZ_hMMP9_Activator
1389. NVS_ENZ_hMsk1
1390. NVS_ENZ_hMsk1_Activator
1391. NVS_ENZ_hNEK2
1392. NVS_ENZ_hNEK2_Activator
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1394. NVS_ENZ_hPAK2_Activator
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1396. NVS_ENZ_hPAK4_Activator
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1398. NVS_ENZ_hPDE10_Activator
1399. NVS_ENZ_hPDE1A1
1400. NVS_ENZ_hPDE1A1_Activator
1401. NVS_ENZ_hPDE1B1
1402. NVS_ENZ_hPDE1B1_Activator
1403. NVS_ENZ_hPDE2A
1404. NVS_ENZ_hPDE2A_Activator
1405. NVS_ENZ_hPDE3A
1406. NVS_ENZ_hPDE3A_Activator
1407. NVS_ENZ_hPDE4A1
1408. NVS_ENZ_hPDE4A1_Activator
1409. NVS_ENZ_hPDE4B1
1410. NVS_ENZ_hPDE4B1_Activator
1411. NVS_ENZ_hPDE4C1
1412. NVS_ENZ_hPDE4C1_Activator
1413. NVS_ENZ_hPDE4D3
1414. NVS_ENZ_hPDE4D3_Activator
1415. NVS_ENZ_hPDE5
1416. NVS_ENZ_hPDE5_Activator
1417. NVS_ENZ_hPDE7A
1418. NVS_ENZ_hPDE7A_Activator
1419. NVS_ENZ_hPDE8A1
1420. NVS_ENZ_hPDE8A1_Activator
1421. NVS_ENZ_hPDE9A2
1422. NVS_ENZ_hPDE9A2_Activator
1423. NVS_ENZ_hPI3Ka
1424. NVS_ENZ_hPI3Ka_Activator
1425. NVS_ENZ_hPKA
1426. NVS_ENZ_hPKA_Activator
1427. NVS_ENZ_hPKCz
1428. NVS_ENZ_hPKCz_Activator
1429. NVS_ENZ_hPKD2
1430. NVS_ENZ_hPKD2_Activator
1431. NVS_ENZ_hPPM1A
1432. NVS_ENZ_hPPM1A_Activator
1433. NVS_ENZ_hPPP1CA
1434. NVS_ENZ_hPPP1CA_Activator
1435. NVS_ENZ_hPPP2CA
1436. NVS_ENZ_hPPP2CA_Activator
1437. NVS_ENZ_hPPP3CA
1438. NVS_ENZ_hPTEN
1439. NVS_ENZ_hPTEN_Activator
1440. NVS_ENZ_hPTPN1
1441. NVS_ENZ_hPTPN1_Activator
1442. NVS_ENZ_hPTPN11
1443. NVS_ENZ_hPTPN11_Activator
1444. NVS_ENZ_hPTPN12
1445. NVS_ENZ_hPTPN12_Activator
1446. NVS_ENZ_hPTPN13
1447. NVS_ENZ_hPTPN13_Activator
1448. NVS_ENZ_hPTPN14
1449. NVS_ENZ_hPTPN14_Activator
1450. NVS_ENZ_hPTPN2
1451. NVS_ENZ_hPTPN2_Activator
1452. NVS_ENZ_hPTPN4
1453. NVS_ENZ_hPTPN4_Activator
1454. NVS_ENZ_hPTPN6
1455. NVS_ENZ_hPTPN6_Activator
1456. NVS_ENZ_hPTPN9
1457. NVS_ENZ_hPTPN9_Activator
1458. NVS_ENZ_hPTPRB
1459. NVS_ENZ_hPTPRB_Activator
1460. NVS_ENZ_hPTPRC
1461. NVS_ENZ_hPTPRC_Activator
1462. NVS_ENZ_hPTPRF
1463. NVS_ENZ_hPTPRF_Activator
1464. NVS_ENZ_hPTPRM
1465. NVS_ENZ_hPTPRM_Activator
1466. NVS_ENZ_hRAF1
1467. NVS_ENZ_hRAF1_Activator
1468. NVS_ENZ_hROCK1
1469. NVS_ENZ_hROCK1_Activator
1470. NVS_ENZ_hSGK1
1471. NVS_ENZ_hSGK1_Activator
1472. NVS_ENZ_hSIRT1
1473. NVS_ENZ_hSIRT1_Activator
1474. NVS_ENZ_hSIRT2
1475. NVS_ENZ_hSIRT2_Activator
1476. NVS_ENZ_hSIRT3
1477. NVS_ENZ_hSIRT3_Activator
1478. NVS_ENZ_hSRC
1479. NVS_ENZ_hSRC_Activator
1480. NVS_ENZ_hSyk
1481. NVS_ENZ_hSyk_Activator
1482. NVS_ENZ_hTie2

1483. NVS_ENZ_hTie2_Activator
1484. NVS_ENZ_hTrkA
1485. NVS_ENZ_hTrkA_Activator
1486. NVS_ENZ_hVEGFR1
1487. NVS_ENZ_hVEGFR1_Activator
1488. NVS_ENZ_hVEGFR2
1489. NVS_ENZ_hVEGFR2_Activator
1490. NVS_ENZ_hVEGFR3
1491. NVS_ENZ_hVEGFR3_Activator
1492. NVS_ENZ_hZAP70
1493. NVS_ENZ_hZAP70_Activator
1494. NVS_ENZ_oCOX1
1495. NVS_ENZ_oCOX1_Activator
1496. NVS_ENZ_oCOX2
1497. NVS_ENZ_oCOX2_Activator
1498. NVS_ENZ_pMTHFR
1499. NVS_ENZ_pMTHFR_Activator
1500. NVS_ENZ_rabI2C
1501. NVS_ENZ_rabI2C_Activator
1502. NVS_ENZ_rACFSKBinding
1503. NVS_ENZ_rACFSKBinding_Activator
1504. NVS_ENZ_rAChE
1505. NVS_ENZ_rAChE_Activator
1506. NVS_ENZ_rCNOS
1507. NVS_ENZ_rCNOS_Activator
1508. NVS_ENZ_rCOMT
1509. NVS_ENZ_rCOMT_Activator
1510. NVS_ENZ_rMAOAC
1511. NVS_ENZ_rMAOAC_Activator
1512. NVS_ENZ_rMAOAP
1513. NVS_ENZ_rMAOAP_Activator
1514. NVS_ENZ_rMAOBC
1515. NVS_ENZ_rMAOBC_Activator
1516. NVS_ENZ_rMAOBP
1517. NVS_ENZ_rMAOBP_Activator
1518. NVS_GPCR_bAdoR_NonSelective
1519. NVS_GPCR_bAT2
1520. NVS_GPCR_bDR_NonSelective
1521. NVS_GPCR_bH1
1522. NVS_GPCR_bNPY_NonSelective
1523. NVS_GPCR_g5HT4
1524. NVS_GPCR_gANPA
1525. NVS_GPCR_gBK2
1526. NVS_GPCR_gH2
1527. NVS_GPCR_gLTB4
1528. NVS_GPCR_gLTD4
1529. NVS_GPCR_gMPeripheral_NonSelective
1530. NVS_GPCR_gOpiateK
1531. NVS_GPCR_h5HT2A
1532. NVS_GPCR_h5HT5A
1533. NVS_GPCR_h5HT6
1534. NVS_GPCR_h5HT7
1535. NVS_GPCR_hAdoRA1
1536. NVS_GPCR_hAdoRA2a
1537. NVS_GPCR_hAdra2A
1538. NVS_GPCR_hAdra2C
1539. NVS_GPCR_hAdrb1
1540. NVS_GPCR_hAdrb2
1541. NVS_GPCR_hAdrb3
1542. NVS_GPCR_hAT1
1543. NVS_GPCR_hC5a
1544. NVS_GPCR_hDRD1
1545. NVS_GPCR_hDRD2s
1546. NVS_GPCR_hDRD4.4
1547. NVS_GPCR_hETA
1548. NVS_GPCR_hETB
1549. NVS_GPCR_hH1
1550. NVS_GPCR_hLTB4_BLT1
1551. NVS_GPCR_hM1
1552. NVS_GPCR_hM2
1553. NVS_GPCR_hM3
1554. NVS_GPCR_hM4
1555. NVS_GPCR_hM5
1556. NVS_GPCR_hNK2
1557. NVS_GPCR_hNPY1
1558. NVS_GPCR_hNPY2
1559. NVS_GPCR_hNTS
1560. NVS_GPCR_hOpiate_D1
1561. NVS_GPCR_hOpiate_mu
1562. NVS_GPCR_hORL1
1563. NVS_GPCR_hPY2
1564. NVS_GPCR_hTXA2
1565. NVS_GPCR_hV1A
1566. NVS_GPCR_mCCKAPeripheral
1567. NVS_GPCR_mCKKBCentral
1568. NVS_GPCR_p5HT2C
1569. NVS_GPCR_r5HT_NonSelective
1570. NVS_GPCR_r5HT1_NonSelective
1571. NVS_GPCR_rabPAF
1572. NVS_GPCR_rAdra1_NonSelective
1573. NVS_GPCR_rAdra1A
1574. NVS_GPCR_rAdra1B
1575. NVS_GPCR_rAdra2_NonSelective
1576. NVS_GPCR_rAdrb_NonSelective
1577. NVS_GPCR_rCRF
1578. NVS_GPCR_rGABBR

1579. NVS_GPCR_rGalanin
1580. NVS_GPCR_rGHB
1581. NVS_GPCR_rH3
1582. NVS_GPCR_rmAdra2B
1583. NVS_GPCR_rmMGluR1
1584. NVS_GPCR_rmMGluR5
1585. NVS_GPCR_rNK1
1586. NVS_GPCR_rNK3
1587. NVS_GPCR_rNTS
1588. NVS_GPCR_rOpiate_NonSelective
1589. NVS_GPCR_rOpiate_NonSelectiveNa
1590. NVS_GPCR_rOXT
1591. NVS_GPCR_rSST
1592. NVS_GPCR_rTRH
1593. NVS_GPCR_rV1
1594. NVS_GPCR_rVIP_NonSelective
1595. NVS_IC_hKhERGCh
1596. NVS_IC_rCaBTZCHL
1597. NVS_IC_rCaChN
1598. NVS_IC_rCaDHPRCh_L
1599. NVS_IC_rKAR
1600. NVS_IC_rKATPCh
1601. NVS_IC_rKCaCh
1602. NVS_IC_rNaCh_site2
1603. NVS_LGIC_bGABAR_Agonist
1604. NVS_LGIC_bGABARa1
1605. NVS_LGIC_bGABARa5
1606. NVS_LGIC_h5HT3
1607. NVS_LGIC_hNNR_NBungSens
1608. NVS_LGIC_rAMPA
1609. NVS_LGIC_rGABAR_NonSelective
1610. NVS_LGIC_rGABARa6
1611. NVS_LGIC_rGluNMDA_Agonist
1612. NVS_LGIC_rGluNMDA_MK801_Agonist
1613. NVS_LGIC_rGlyRStrySens
1614. NVS_LGIC_rNNR_BungSens
1615. NVS_MP_hPBR
1616. NVS_MP_rPBR
1617. NVS_NR_bPR
1618. NVS_NR_hCAR_Agonist
1619. NVS_NR_hCAR_Antagonist
1620. NVS_NR_hGR
1621. NVS_NR_hPR
1622. NVS_NR_hPXR
1623. NVS_NR_hRAR_Antagonist
1624. NVS_NR_hRARa_Agonist
1625. NVS_NR_rMR
1626. NVS_OR_gSIGMA_NonSelective
1627. NVS_OR_hFKBP12
1628. NVS_TR_gDAT
1629. NVS_TR_hAdoT
1630. NVS_TR_hDAT
1631. NVS_TR_hNET
1632. NVS_TR_hSERT
1633. NVS_TR_rAdoT
1634. NVS_TR_rNET
1635. NVS_TR_rSERT
1636. NVS_TR_rVMAT2

1637. OT_Apoptosis_PARP1_1440_inh
1638. OT_Apoptosis_PARP1_1440_sti
1639. OT_ATR_Chk1Cdc25C_0480_inh
1640. OT_ATR_Chk1Cdc25C_0480_sti
1641. OT_ATR_Chk1Cdc25C_1440_inh
1642. OT_ATR_Chk1Cdc25C_1440_sti
1643. OT_ATR_Chk1Cdc25CCPT_1440_inh
1644. OT_ATR_Chk1Cdc25CCPT_1440_sti
1645. OT_b2AR_b2ARbARR2_0090_inh
1646. OT_b2AR_b2ARbARR2_0090_sti
1647. OT_b2AR_b2ARbARR2_0180_inh
1648. OT_b2AR_b2ARbARR2_0180_sti
1649. OT_b2AR_b2ARbARR2INN_0240_inh
1650. OT_b2AR_b2ARbARR2INN_0240_sti
1651. OT_CellCycle_Cdc2Cdc25C_0480_inh
1652. OT_CellCycle_Cdc2Cdc25C_0480_sti
1653. OT_CellCycle_Cdc2Cdc25C_1440_inh
1654. OT_CellCycle_Cdc2Cdc25C_1440_sti
1655. OT_CXCR4_CXCR4bARR2_0090_inh
1656. OT_CXCR4_CXCR4bARR2_0090_sti
1657. OT_CXCR4_CXCR4bARR2_0480_inh
1658. OT_CXCR4_CXCR4bARR2_0480_sti
1659. OT_CXCR4_CXCR4bARR2CXCL12_0240_inh
1660. OT_CXCR4_CXCR4bARR2CXCL12_0240_sti
1661. OT_ERStress_GRP78_1440_inh
1662. OT_ERStress_GRP78_1440_sti
1663. OT_H2AX_gH2AX_1440_inh
1664. OT_H2AX_gH2AX_1440_sti
1665. OT_HEK293T_LDH_2880_inh
1666. OT_HEK293T_LDH_2880_sti
1667. OT_Hepatocyte_LDH_2880_inh
1668. OT_Hepatocyte_LDH_2880_sti
1669. OT_NegCtrl_EmptyReporter_0240_inh
1670. OT_NegCtrl_EmptyReporter_0240_sti
1671. OT_NegCtrl_EmptyReporter_0480_inh
1672. OT_NegCtrl_EmptyReporter_0480_sti
1673. OT_NegCtrl_EmptyReporter_0960_inh
1674. OT_NegCtrl_EmptyReporter_0960_sti
1675. OT_NegCtrl_EmptyReporter_1440_inh
1676. OT_NegCtrl_EmptyReporter_1440_sti
1677. OT_NegCtrl_Fluorescence_0240_inh
1678. OT_NegCtrl_Fluorescence_0240_sti
1679. OT_NegCtrl_Fluorescence_0480_inh
1680. OT_NegCtrl_Fluorescence_0480_sti
1681. OT_NegCtrl_Fluorescence_0960_inh
1682. OT_NegCtrl_Fluorescence_0960_sti
1683. OT_NegCtrl_Fluorescence_1440_inh
1684. OT_NegCtrl_Fluorescence_1440_sti
1685. OT_NFKb_p50p65_0480_inh
1686. OT_NFKb_p50p65_0480_sti
1687. OT_NFKb_p50p65_1440_inh
1688. OT_NFKb_p50p65_1440_sti
1689. OT_NFKb_p50p65TNFa_0090_inh
1690. OT_NFKb_p50p65TNFa_0090_sti
1691. OT_NFKb_p50p65TNFa_0480_inh
1692. OT_NFKb_p50p65TNFa_0480_sti
1693. OT_NURR1_NURR1RXRa_0480
1694. OT_NURR1_NURR1RXRa_1440
1695. OT_p38MAPK_Mnk1p38_0480_inh
1696. OT_p38MAPK_Mnk1p38_0480_sti
1697. OT_p38MAPK_Mnk1p38_1440_inh
1698. OT_p38MAPK_Mnk1p38_1440_sti
1699. OT_p53_Mdm2p53_0480_inh
1700. OT_p53_Mdm2p53_0480_sti
1701. OT_p53_Mdm2p53_1440_inh
1702. OT_p53_Mdm2p53_1440_sti
1703. OT_p53_Pin1p53_0480_inh
1704. OT_p53_Pin1p53_0480_sti
1705. OT_p53_Pin1p53_1440_inh
1706. OT_p53_Pin1p53_1440_sti
1707. OT_p53_Pin1p53CPT_1440_inh
1708. OT_p53_Pin1p53CPT_1440_sti
1709. OT_PI3K_Akt1PDk1_0090_inh
1710. OT_PI3K_Akt1PDk1_0090_sti
1711. OT_PI3K_Akt1PDk1_0480_inh
1712. OT_PI3K_Akt1PDk1_0480_sti
1713. OT_PI3K_Akt1PDk1_1440_inh
1714. OT_PI3K_Akt1PDk1_1440_sti
1715. OT_RhoRock_Limk2Cofilin1_0480_inh
1716. OT_RhoRock_Limk2Cofilin1_0480_sti
1717. OT_RhoRock_Limk2Cofilin1_1440_inh
1718. OT_RhoRock_Limk2Cofilin1_1440_sti

1719. STEMINA_H9_C13Cystine_area_dn
1720. STEMINA_H9_C13Cystine_area_up
1721. STEMINA_H9_C13Ornithine_area_dn
1722. STEMINA_H9_C13Ornithine_area_up
1723. STEMINA_H9_Cystine_area_dn
1724. STEMINA_H9_Cystine_area_up
1725. STEMINA_H9_CystineISnorm_dn
1726. STEMINA_H9_CystineISnorm_perc_dn
1727. STEMINA_H9_CystineISnorm_perc_up
1728. STEMINA_H9_CystineISnorm_up
1729. STEMINA_H9_ORNCYSSISnorm_Prediction_dn
1730. STEMINA_H9_ORNCYSSISnorm_Prediction_up
1731. STEMINA_H9_ORNCYSSISnorm_RATIO_dn
1732. STEMINA_H9_ORNCYSSISnorm_RATIO_up
1733. STEMINA_H9_Ornithine_area_dn
1734. STEMINA_H9_Ornithine_area_up
1735. STEMINA_H9_OrnithineISnorm_dn
1736. STEMINA_H9_OrnithineISnorm_perc_dn
1737. STEMINA_H9_OrnithineISnorm_perc_up
1738. STEMINA_H9_OrnithineISnorm_up
1739. STEMINA_H9_Viability_Norm

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1740. Tanguay_ZF_120hpf_ActivityScore
1741. Tanguay_ZF_120hpf_AXIS_up
1742. Tanguay_ZF_120hpf_BRAI_up
1743. Tanguay_ZF_120hpf_CFIN_up
1744. Tanguay_ZF_120hpf_CIRC_up
1745. Tanguay_ZF_120hpf_EYE_up
1746. Tanguay_ZF_120hpf_JAW_up
1747. Tanguay_ZF_120hpf_MORT_up
1748. Tanguay_ZF_120hpf_NC_up
1749. Tanguay_ZF_120hpf_OTIC_up
1750. Tanguay_ZF_120hpf_PE_up
1751. Tanguay_ZF_120hpf_PFIN_up
1752. Tanguay_ZF_120hpf_PIG_up
1753. Tanguay_ZF_120hpf_SNOU_up
1754. Tanguay_ZF_120hpf_SOMI_up
1755. Tanguay_ZF_120hpf_SWIM_up
1756. Tanguay_ZF_120hpf_TR_up
1757. Tanguay_ZF_120hpf_TRUN_up
1758. Tanguay_ZF_120hpf_YSE_up

1759. TOX21_AChE_Inhibition_Colormetric
1760. TOX21_AChE_Inhibition_Fluor
1761. TOX21_AhR_LUC_Agonist
1762. TOX21_AhR_LUC_Agonist_viability
1763. TOX21_AP1_BLA_Agonist_ch1
1764. TOX21_AP1_BLA_Agonist_ch2
1765. TOX21_AP1_BLA_Agonist_ratio
1766. TOX21_AP1_BLA_Agonist_viability
1767. TOX21_AR_BLA_Agonist_ch1
1768. TOX21_AR_BLA_Agonist_ch2
1769. TOX21_AR_BLA_Agonist_ratio
1770. TOX21_AR_BLA_Antagonist_ch1
1771. TOX21_AR_BLA_Antagonist_ch2
1772. TOX21_AR_BLA_Antagonist_ratio
1773. TOX21_AR_BLA_Antagonist_viability
1774. TOX21_AR_LUC_MDAKB2_Agonist
1775. TOX21_AR_LUC_MDAKB2_Agonist2
1776. TOX21_AR_LUC_MDAKB2_Agonist2_viability
1777. TOX21_AR_LUC_MDAKB2_Antagonist
1778. TOX21_AR_LUC_MDAKB2_Antagonist_viability
1779. TOX21_AR_LUC_MDAKB2_Antagonist2
1780. TOX21_AR_LUC_MDAKB2_Antagonist2_viability
1781. TOX21_ARE_BLA_Agonist_ch1
1782. TOX21_ARE_BLA_Agonist_ch2
1783. TOX21_ARE_BLA_agonist_ratio
1784. TOX21_ARE_BLA_agonist_viability
1785. TOX21_Aromatase_Inhibition
1786. TOX21_Aromatase_Inhibition_viability
1787. TOX21_AutoFluor_HEK293_Cell_blue
1788. TOX21_AutoFluor_HEK293_Cell_green
1789. TOX21_AutoFluor_HEK293_Cell_red
1790. TOX21_AutoFluor_HEK293_Media_blue
1791. TOX21_AutoFluor_HEK293_Media_green
1792. TOX21_AutoFluor_HEK293_Media_red
1793. TOX21_AutoFluor_HEPG2_Cell_blue
1794. TOX21_AutoFluor_HEPG2_Cell_green
1795. TOX21_AutoFluor_HEPG2_Cell_red
1796. TOX21_AutoFluor_HEPG2_Media_blue
1797. TOX21_AutoFluor_HEPG2_Media_green
1798. TOX21_AutoFluor_HEPG2_Media_red
1799. TOX21_CAR_Agonist
1800. TOX21_CAR_Agonist_viabillity
1801. TOX21_CAR_Antagonist
1802. TOX21_CAR_Antagonist_viability
1803. TOX21_CASP3_CHO
1804. TOX21_CASP3_CHO_viability
1805. TOX21_CASP3_HEPG2
1806. TOX21_CASP3_HEPG2_viability
1807. TOX21_DT40
1808. TOX21_DT40_100
1809. TOX21_DT40_657
1810. TOX21_ELG1_LUC_Agonist
1811. TOX21_ELG1_LUC_Agonist_viability
1812. TOX21_ERa_BLA_Agonist_ch1
1813. TOX21_ERa_BLA_Agonist_ch2
1814. TOX21_ERa_BLA_Agonist_ratio
1815. TOX21_ERa_BLA_Antagonist_ch1
1816. TOX21_ERa_BLA_Antagonist_ch2
1817. TOX21_ERa_BLA_Antagonist_ratio
1818. TOX21_ERa_BLA_Antagonist_viability
1819. TOX21_ERa_LUC_BG1_Agonist
1820. TOX21_ERa_LUC_BG1_Agonist2
1821. TOX21_ERa_LUC_BG1_Agonist2_viability
1822. TOX21_ERa_LUC_BG1_Antagonist
1823. TOX21_ERa_LUC_BG1_Antagonist_viability
1824. TOX21_ERa_LUC_BG1_Antagonist2
1825. TOX21_ERa_LUC_BG1_Antagonist2_viability
1826. TOX21_ERb_BLA_Agonist_ch1
1827. TOX21_ERb_BLA_Agonist_ch2
1828. TOX21_ERb_BLA_Agonist_ratio
1829. TOX21_ERb_BLA_Agonist_viability
1830. TOX21_ERb_BLA_Antagonist_ch1
1831. TOX21_ERb_BLA_Antagonist_ch2
1832. TOX21_ERb_BLA_Antagonist_ratio
1833. TOX21_ERb_BLA_Antagonist_viability
1834. TOX21_ERR_Agonist
1835. TOX21_ERR_Antagonist
1836. TOX21_ERR_viability
1837. TOX21_ESRE_BLA_ch1
1838. TOX21_ESRE_BLA_ch2
1839. TOX21_ESRE_BLA_ratio
1840. TOX21_ESRE_BLA_viability

1841. TOX21_FXR_BLA_agonist_ch1
1842. TOX21_FXR_BLA_agonist_ch2
1843. TOX21_FXR_BLA_agonist_ratio
1844. TOX21_FXR_BLA_agonist_viability
1845. TOX21_FXR_BLA_Antagonist_ch1
1846. TOX21_FXR_BLA_Antagonist_ch2
1847. TOX21_FXR_BLA_antagonist_ratio
1848. TOX21_FXR_BLA_antagonist_viability
1849. TOX21_GR_BLA_Agonist_ch1
1850. TOX21_GR_BLA_Agonist_ch2
1851. TOX21_GR_BLA_Agonist_ratio
1852. TOX21_GR_BLA_Antagonist_ch1
1853. TOX21_GR_BLA_Antagonist_ch2
1854. TOX21_GR_BLA_Antagonist_ratio
1855. TOX21_GR_BLA_Antagonist_viability
1856. TOX21_H2AX_HTRF_CHO_Agonist_ch1
1857. TOX21_H2AX_HTRF_CHO_Agonist_ch2
1858. TOX21_H2AX_HTRF_CHO_Agonist_ratio
1859. TOX21_H2AX_HTRF_CHO_viability
1860. TOX21_HDAC_Inhibition
1861. TOX21_HDAC_Inhibition_viability
1862. TOX21_HRE_BLA_Agonist_ch1
1863. TOX21_HRE_BLA_Agonist_ch2
1864. TOX21_HRE_BLA_Agonist_ratio
1865. TOX21_HRE_BLA_Agonist_viability
1866. TOX21_HSE_BLA_agonist_ch1
1867. TOX21_HSE_BLA_agonist_ch2
1868. TOX21_HSE_BLA_agonist_ratio
1869. TOX21_HSE_BLA_agonist_viability
1870. TOX21_LUC_Biochem
1871. TOX21_MMP_fitc
1872. TOX21_MMP_ratio_down
1873. TOX21_MMP_ratio_up
1874. TOX21_MMP_rhodamine
1875. TOX21_MMP_viability
1876. TOX21_NFkB_BLA_agonist_ch1
1877. TOX21_NFkB_BLA_agonist_ch2
1878. TOX21_NFkB_BLA_agonist_ratio
1879. TOX21_NFkB_BLA_agonist_viability
1880. TOX21_p53_BLA_p1_ch1
1881. TOX21_p53_BLA_p1_ch2
1882. TOX21_p53_BLA_p1_ratio
1883. TOX21_p53_BLA_p1_viability
1884. TOX21_p53_BLA_p2_ch1
1885. TOX21_p53_BLA_p2_ch2
1886. TOX21_p53_BLA_p2_ratio
1887. TOX21_p53_BLA_p2_viability
1888. TOX21_p53_BLA_p3_ch1
1889. TOX21_p53_BLA_p3_ch2
1890. TOX21_p53_BLA_p3_ratio
1891. TOX21_p53_BLA_p3_viability
1892. TOX21_p53_BLA_p4_ch1
1893. TOX21_p53_BLA_p4_ch2
1894. TOX21_p53_BLA_p4_ratio
1895. TOX21_p53_BLA_p4_viability
1896. TOX21_p53_BLA_p5_ch1
1897. TOX21_p53_BLA_p5_ch2
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1899. TOX21_p53_BLA_p5_viability
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1901. TOX21_PGC_ERR_Antagonist
1902. TOX21_PGC_ERR_viability
1903. TOX21_PPARd_BLA_agonist_ch1
1904. TOX21_PPARd_BLA_agonist_ch2
1905. TOX21_PPARd_BLA_agonist_ratio
1906. TOX21_PPARd_BLA_Agonist_viability
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1926. TOX21_PR_BLA_Followup_Agonist_ch1
1927. TOX21_PR_BLA_Followup_Agonist_ch2
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1929. TOX21_PR_BLA_Followup_Agonist_viability
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1931. TOX21_PR_BLA_Followup_Antagonist_ch2
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1935. TOX21_PR_LUC_Followup_Agonist_viability
1936. TOX21_PR_LUC_Followup_Antagonist

1937. TOX21_PR_LUC_Followup_Antagonist_viability
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1946. TOX21_RT_HEK293_FLO_00hr_viability
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1948. TOX21_RT_HEK293_FLO_16hr_viability
1949. TOX21_RT_HEK293_FLO_24hr_viability
1950. TOX21_RT_HEK293_FLO_32hr_viability
1951. TOX21_RT_HEK293_FLO_40hr_viability
1952. TOX21_RT_HEK293_GLO_00hr_viability
1953. TOX21_RT_HEK293_GLO_08hr_viability
1954. TOX21_RT_HEK293_GLO_16hr_viability
1955. TOX21_RT_HEK293_GLO_24hr_viability
1956. TOX21_RT_HEK293_GLO_32hr_viability
1957. TOX21_RT_HEK293_GLO_40hr_viability
1958. TOX21_RT_HEPG2_FLO_00hr_ctrl_viability
1959. TOX21_RT_HEPG2_FLO_08hr_viability
1960. TOX21_RT_HEPG2_FLO_16hr_ctrl_viability
1961. TOX21_RT_HEPG2_FLO_24hr_viability
1962. TOX21_RT_HEPG2_FLO_32hr_ctrl_viability
1963. TOX21_RT_HEPG2_FLO_40hr_ctrl_viability
1964. TOX21_RT_HEPG2_GLO_00hr_ctrl_viability
1965. TOX21_RT_HEPG2_GLO_08hr_ctrl_viability
1966. TOX21_RT_HEPG2_GLO_16hr_ctrl_viability
1967. TOX21_RT_HEPG2_GLO_24hr_ctrl_viability
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1972. TOX21_RXR_BLA_Agonist_ratio
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1975. TOX21_SBE_BLA_Agonist_ch2
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1979. TOX21_SBE_BLA_Antagonist_ch2
1980. TOX21_SBE_BLA_Antagonist_ratio
1981. TOX21_SBE_BLA_Antagonist_viability
1982. TOX21_SSH_3T3_GLI3_Agonist
1983. TOX21_SSH_3T3_GLI3_Agonist_viability
1984. TOX21_SSH_3T3_GLI3_Antagonist
1985. TOX21_SSH_3T3_GLI3_Antagonist_viability
1986. TOX21_TR_COA_Antagonist_Followup_ch1
1987. TOX21_TR_COA_Antagonist_Followup_ch2
1988. TOX21_TR_COA_Antagonist_Followup_ratio
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1999. TOX21_TR_RXR_BLA_Antagonist_Followup_ch2
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2001. TOX21_TR_RXR_BLA_Antagonist_Followup_viability
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2008. TOX21_TRB_BLA_Agonist_Followup_ch1
2009. TOX21_TRB_BLA_Agonist_Followup_ch2
2010. TOX21_TRB_BLA_Agonist_Followup_ratio
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2013. TOX21_TRB_BLA_Antagonist_Followup_ch2
2014. TOX21_TRB_BLA_Antagonist_Followup_ratio
2015. TOX21_TRB_BLA_Antagonist_Followup_viability
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2021. TOX21_TRB_COA_Antagonist_Followup_ratio
2022. TOX21_TRHR_HEK293_Agonist
2023. TOX21_TRHR_HEK293_Antagonist
2024. TOX21_TSHR_Agonist_ch1
2025. TOX21_TSHR_Agonist_ch2
2026. TOX21_TSHR_Agonist_ratio
2027. TOX21_TSHR_Antagonist_ch1
2028. TOX21_TSHR_Antagonist_ch2
2029. TOX21_TSHR_Antagonist_ratio
2030. TOX21_TSHR_wt_ch1
2031. TOX21_TSHR_wt_ch2
2032. TOX21_TSHR_wt_ratio

2033. TOX21_VDR_BLA_agonist_ch1
2034. TOX21_VDR_BLA_agonist_ch2
2035. TOX21_VDR_BLA_agonist_ratio
2036. TOX21_VDR_BLA_Agonist_viability
2037. TOX21_VDR_BLA_Antagonist_ch1
2038. TOX21_VDR_BLA_Antagonist_ch2
2039. TOX21_VDR_BLA_antagonist_ratio
2040. TOX21_VDR_BLA_antagonist_viability

2041. Vala_A1_CellCount_dn
2042. Vala_A1_K2_ADFPvLipid_dn
2043. Vala_A1_K2_ADFPvLipid_up
2044. Vala_A1_LipidDropletDiam_dn
2045. Vala_A1_LipidDropletDiam_up
2046. Vala_A1_LipidPxlInt_dn
2047. Vala_A1_LipidPxlInt_up
2048. Vala_A1_PCC_ADFPvLipid_dn
2049. Vala_A1_PCC_ADFPvLipid_up
2050. Vala_A1_PCC_PerilipinvLipid_dn
2051. Vala_A1_PCC_PerilipinvLipid_up
2052. Vala_A1_PctDifferenCells_dn
2053. Vala_A1_PctDifferenCells_up
2054. Vala_A2_CellCount_dn
2055. Vala_A2_K1_ADFPvLipid_dn
2056. Vala_A2_K1_ADFPvLipid_up
2057. Vala_A2_K2_LipidvADFP_dn
2058. Vala_A2_K2_LipidvADFP_up
2059. Vala_A2_LipidDropletDiam_dn
2060. Vala_A2_LipidDropletDiam_up
2061. Vala_A2_LipidPxlInt_dn
2062. Vala_A2_LipidPxlInt_up
2063. Vala_A2_PCC_LipidvPerilipin_dn
2064. Vala_A2_PCC_LipidvPerilipin_up
2065. Vala_A2_PctDifferenCells_dn
2066. Vala_A2_PctDifferenCells_up
2067. Vala_BC1_CellCount_dn
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2069. Vala_BC1_PCC_betacateninvNucleus_up
2070. Vala_ECAD_CellCount_dn
2071. Vala_ECAD_ECADCellInt_dn
2072. Vala_ECAD_ECADCellInt_up
2073. Vala_ECAD_ECADMembraneInt_dn
2074. Vala_ECAD_ECADMembraneInt_up
2075. Vala_ECAD_TSA_dn
2076. Vala_ECAD_TSA_up
2077. Vala_L2_CellCount_dn
2078. Vala_L2_LipidDropletDiam_dn
2079. Vala_L2_LipidDropletDiam_up
2080. Vala_L2_PCC_pHSLvLipid_dn
2081. Vala_L2_PCC_pHSLvLipid_up
2082. Vala_L2_PCC_pPerilipinvLipid_dn
2083. Vala_L2_PCC_pPerilipinvLipid_up
2084. Vala_L2_PctDifferenCells_dn
2085. Vala_L2_PctDifferenCells_up
2086. Vala_LD1_CellCount_dn
2087. Vala_LD1_LipidDropletArea_dn
2088. Vala_LD1_LipidDropletArea_up
2089. Vala_LD1_LipidDropletDiam_dn
2090. Vala_LD1_LipidDropletDiam_up
2091. Vala_LD1_LipidPxlInt_dn
2092. Vala_LD1_LipidPxlInt_up
2093. Vala_LD1_M2_ADFPvLipid_m_dn
2094. Vala_LD1_M2_ADFPvLipid_m_up
2095. Vala_LD1_M2_ADFPvLipid_md_dn
2096. Vala_LD1_M2_ADFPvLipid_md_up
2097. Vala_LD2_CellCount_dn
2098. Vala_LD2_LipidDropletDiam_dn
2099. Vala_LD2_LipidDropletDiam_up
2100. Vala_LD2_LipidPxlInt_dn
2101. Vala_LD2_LipidPxlInt_up
2102. Vala_LD2_M2_ADFPvLipid_dn
2103. Vala_LD2_M2_ADFPvLipid_up
2104. Vala_LD2_PCC_ADFPvlipid_dn
2105. Vala_LD2_PCC_ADFPvlipid_up
2106. Vala_MEF1_CellCount_dn
2107. Vala_MEF1_Flk1Int_dn
2108. Vala_MEF1_Flk1Int_up
2109. Vala_MEF1_MOC_NucleusFoxA2_dn
2110. Vala_MEF1_MOC_NucleusFoxA2_up
2111. Vala_MEF1_PCC_NucleusvFlk1_dn
2112. Vala_MEF1_PCC_NucleusvFlk1_up
2113. Vala_MEF1_PCC_NucleusvFoxA2_dn
2114. Vala_MEF1_PCC_NucleusvFoxA2_up
2115. Vala_NO1_CellCount_dn
2116. Vala_NO1_M1_PrePostSynapticImage_dn
2117. Vala_NO1_M1_PrePostSynapticImage_up
2118. Vala_NO1_M2_PrePostNeuriteImage_dn
2119. Vala_NO1_M2_PrePostNeuriteImage_up
2120. Vala_NO1_NeuriteArea_dn
2121. Vala_NO1_NeuriteArea_up
2122. Vala_PBCD_CellCount_dn
2123. Vala_PBCD_FractionCellsGFPabvbgd_dn
2124. Vala_PBCD_FractionCellsGFPabvbgd_up
2125. Vala_PBCD_GFPNucleusInt_dn
2126. Vala_PBCD_GFPNucleusInt_up

2127. Vala_SCP1_CellCount_dn
2128. Vala_SCP1_PCC_Oct3vNucleus_dn
2129. Vala_SCP1_PCC_Oct3vNucleus_up
2130. Vala_VECAD_CellCount_dn
2131. Vala_VECAD_TSA_dn
2132. Vala_VECAD_TSA_up
2133. Vala_VECAD_TSAMiMm_dn
2134. Vala_VECAD_TSAMiMm_up
2135. Vala_VECAD_WholeCellInt_dn
2136. Vala_VECAD_WholeCellInt_up

ASSAY DOCUMENTATION

Endocrine Assays

ACEA_T47D_80hr_Positive

Assay Title: ACEA 80-hr T47-D Human Breast Cell Proliferation Assay

Overview

Assay Summary:

One possible effect of endocrine disrupting chemicals is increased cell growth through perturbation of endocrine pathways linked to cell cycle regulation. Activation of the estrogen receptor (ER) signaling pathway, for example, is one possible mechanism that underlies cell proliferation in hormonally sensitive tissues such as mammary and endometrial tissue. The role of steroid hormones in the regulation of some mammary tumors has been well established (Russo and Russo 2006, Yager and Davidson 2006) and has motivated the development of estrogen pathway-based chemotherapeutics. This assay was designed to identify those chemicals in the ToxCast chemical library with the potential to affect cell growth by activating the estrogen receptor-mediated cell proliferation pathway. These impacts were observed by monitoring changes in electrical impedance on the surface of an electronic cell culture growth plate (E-plates) following 80-hour incubation with test chemicals.

Assay Definition

Assay Throughput:

The assay is conducted on 96-well plates with each plate containing positive controls for proliferation (17β-estradiol) and cytotoxicity (MG132), negative controls (assay media, RPMI 1640), and two concentrations (0.5% and 0.125%) of DMSO solvent controls. Following a 24-hour incubation period, the cells are exposed to test chemicals for 80 hours and response is monitored no less than once per hour.

Experimental System:

T-47D human breast carcinoma ductal cell line, originally derived in 1974 from pleural effusion of a 57-year-old patient, which exhibits epithelial-like morphology (Horwitz et al. 1978, Keydar et al. 1979).

Xenobiotic Biotransformation Potential:

T-47D cells contain specific high affinity receptors for estradiol, progesterone, glucocorticoid and androgen (Horwitz et al. 1978). Some potential for P450 mediated metabolism is present, e.g. CYP1A1, CYP1A2, CYP1B1 (Angus et al. 1999, Hevir et al. 2011, MacPherson and Matthews 2010, Spink et al. 2002, Spink et al. 1998), CYP2B6 (Lo et al. 2010), CYP3A4 (Nagaoka et al. 2006) and CYP2C8 (Mitra et al. 2011), as well as some experimental evidence for the capacity to retain expression of some phase II metabolizing enzymes, e.g., UGTs (Harrington et al. 2006, Hevir et al. 2011), GSTs (Hevir et al. 2011) and sulphotransferases (e.g., SULT1A3(Miki et al. 2006), SULT1E1, SULT2B1 (Hevir et al. 2011)).

Basic Procedure:

Materials

	Product	Source	Cat. No.
Cells	T-47D	ATCC	HTB-133
Growth media	RPMI1640	Hyclone	SH30027FS
Growth media serum	10% FBS	Hyclone	SH3007103
Test media	RPMI 1640	Gibco.	11835030
Test media serum	10% charcoal stripped FBS	Hyclone	SH3006803HI
Positive control	17β-estradiol	Tocris	2824
Reference compound	dexamethasone	Sigma-Aldrich	D1756
Reference compound	hydrocortisone	Sigma-Aldrich	H4001
Reference compound	progesterone	Sigma-Aldrich	P8783
Reference compound	aldosterone	Sigma-Aldrich	A9477
Reference compound	T3 (3,3',5-Triiodo-L-thyronine sodium salt)	Sigma-Aldrich	T6397
Reference compound	T4 (thyroxine)	Sigma-Aldrich	T2376

Protocols

T-47D cells purchased from American Tissue Culture Collection (ATCC) were maintained in RPMI1640 media supplemented with 10% characterized fetal bovine serum (FBS) until testing. Before screening, cells were preconditioned in assay medium: phenol red-free RPMI1640 supplemented with 10% charcoal-stripped FBS. Cells were then detached and seeded in 96-well E-Plates in assay medium. After overnight monitoring of growth once every hour, chemicals were added to T-47D cells and remained in the medium until the end of the experiment. Each chemical in the ToxCast library was tested in an 8-point, 1:4 serial dilution series starting at a maximum final concentration of 100 µM and was tested in duplicate using two separate E-plates for each dilution series. A maximum starting concentration of 0.5% DMSO was present in the 100 µM chemical samples and subsequent dilutions used a final concentration of 0.125% DMSO. Positive controls (MG132 for cytotoxicity and 17β-estradiol for proliferation) and a negative control (assay media) were tested in quadruplicate on each testing plate along with 0.5% and 0.125% DMSO tested in duplicates on each plate to serve as solvent controls for the highest concentration of testing chemicals and all lower dilutions, respectively. Reference chemicals were tested with 8 concentrations with 1:5 serial dilutions. The xCELLigence system Multi-E-Plate stations employing real-time cell analysis (RTCA) were used to measure cellular responses recorded once every 5 min for the first 5 h, and once every hour for an additional 100h.

Proprietary Elements:

Assay is non-proprietary; xCELLigence RTCA software and biosensor technology are available from ACEA Biosciences, Inc. and T-47D cells are commercially available from American Type Culture Collection (ATCC HTB-133) with signed Material Transfer Agreement (MTA).

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to activate endogenous estrogenic signaling pathways, and is intended to provide information

on a large number of diverse chemicals. Cell proliferation may result from both estrogenic pathways and non-estrogenic pathways so results from this assay in isolation do not ensure estrogenic activity for a test chemical. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

Assay References

Assay Source Contact Information:

ACEA Biosciences

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Fax: +1 858-724-0927

info@aceabio.com

Assay Publication Year:

2006

Assay Publication:

Xing, J. Z., Zhu, L., Gabos, S., & Xie, L. (2006). "Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity". Toxicol In Vitro 20(6), 995-1004. (PMID: 16481145)

Method Updates / Confirmatory Studies:

None Reported

Assay Component Descriptions

Assay Objectives:

The ACEA T47D 80-hour Positive assay exposed human breast carcinoma cell (T-47D) cultures to the ToxCast library of diverse environmental chemicals using an eight-point, 1:4 dilution series concentration-response format (starting at a maximum final concentration of 100µM), using MG132 (cytotoxicity) and Estradiol (E2) (proliferation) as positive controls and assay media and DMSO as a negative control and solvent control, respectively. All control chemicals were tested in quadruplicate on each plate.

The ACEA_T47D ER assay analyzed changes in cell adhesion and morphology at the electrode: solution interface (located on the bottom of 96-well E-plate culture wells) using electronic microsensors. Changes

in electrical impedance were monitored in real-time at the plate surface to investigate the potential activation of the estrogen signaling pathway and subsequent increases in growth or changes in cell structure following 80-hour incubation with the test chemicals. The electrical signal produced by the experimental system can be used to detect changes in cell number, morphology and adhesion which occur in response to xenoestrogenic activation of ER-mediated pathways, and concentration-response curves were modeled for each chemical to determine half-maximal activity levels.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogenic signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The activity of estrogenic chemicals is generally probed in vitro by monitoring ligand-binding in experimental systems, however estrogenic potency is also a function of interaction with transcriptional machinery and other signaling pathways. This assay was designed to identify chemical perturbagens which can affect a cell proliferation response in human breast carcinoma cells by acting as xenoestrogenic compounds which impact estrogen signaling pathways. While cell proliferation rates can be altered via multiple pathways, growth responses in T47D cells are considered to be particularly reliable indicators of estrogenic activation.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds which potentially affect endocrine systems in exposed populations by interacting with estrogen receptor mediated signaling pathways. There is strong evidence that estrogen receptor activity in early life is a molecular initiating event (MIE) in a developing Adverse Outcome Pathways (AOP) leading to breast cancer in both animal and human models and to endometrial carcinoma in the mouse, and ER agonism is the leading to reproductive dysfunction in oviparous vertebrates (AOPs under development), and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (under development). ER antagonism has strong evidence as the MIE for an AOP describing reduction of vitellogenin synthesis in liver (under review), which can lead to reduced cumulative fecundity in repeat-spawning fish species. Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER interference in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Xiao, C., Lachance, B., Sunahara, G., & Luong, J. H. (2002). "Assessment of cytotoxicity using electric cell-substrate impedance sensing: concentration and time response function approach". Analytical Chemistry 74(22), 5748-5753. (PMID: 12463358)

Assay Quality Statistics:

Neutral control well median response value, by plate: 1.66

Neutral control median absolute deviation, by plate:	0.11
Positive control well median response value, by plate:	3.00
Positive control well median absolute deviation, by plate:	0.19
Negative control well median, by plate:	0.03
Negative control well median absolute deviation value, by plate:	0.06
Z' (median across all plates, using positive control wells):	0.31
Z' (median across all plates, using negative control wells):	0.69
SSMD (median across all plates, using positive control wells):	6.00
SSMD (median across all plates, using negative control wells):	-13.00
Signal-to-noise (median across all plates, using positive control wells):	12.94
Signal-to-noise (median across all plates, using negative control wells):	-15.00
Signal-to-background (median across all plates, using positive control wells):	1.82
Signal-to-background (median across all plates, using negative control wells):	0.02
CV (median across all plates):	0.07

Assay Endpoint Descriptions

Data Interpretation

Biological Response:

Increase cell proliferation in response to xenoestrogenic interference with ER-mediated pathways as measured by monitoring electrical impedance at the cell-plate interface.

Analytical Elements:

Data were collected from the xCELLigence system which converts raw impedance values into the Cell Index (CI) value; this is a measure of adhesion where CI = (impedance at time point n – impedance in the absence of cells)/nominal impedance value. These data were then converted to a Normalized Cell Index according to the equation NCI(Ti) = [CI(Ti)]/[CI(Tk)], {i = 1,2,3,….N where CI(Tk) is the last time point before chemical addition, CI(Ti) is the cell index at the i-th measured time point, and N is the total number of time points. Data were grouped by chemical and smoothed to combine replicates using a simple moving average (as the replicates were assessed in duplicate on separate plates so the time points were not identical). DMSO controls were considered as baseline for activity, and 17β-Estradiol was used as a positive control and 100% activity for all the test chemicals on that plate. If a chemical sample was run on two different plates, then the maximum NCI values for the positive and negative controls were averaged. Concentration response curves were generated using smoothed NCI values and all statistical

analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive

ATG_ERE_CIS_up

ATG_ERa_TRANS_up

NVS_NR_bER

NVS_NR_hER

NVS_NR_mERa

OT_ER_ERaERa_0480

OT_ER_ERaERa_1440

OT_ER_ERaERb_0480

OT_ER_ERaERb_1440

OT_ERa_ERELUC_AG_1440

OT_ERa_ERELUC_ANT_1440

OT_ERa_EREGFP_0120

OT_ERa_EREGFP_0480

Tox21_ERa_BLA_Agonist_ratio

Tox21_ERa_BLA_Antagonist_ratio

Tox21_ERa_LUC_BG1_Agonist

Tox21_ERa_LUC_BG1_Antagonist

Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 8

Target (nominal) number of replicates: 2

Standard minimum concentration tested: 0.39 µM

Standard maximum concentration tested: 200 µM

Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 4.77

The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 23.86

Reference Chemicals / Predictive Capacity:

ER reference chemicals (list adapted from OECD Test Guideline No. 457, as according to methods described by Judson et al. 2015 (Judson et al. 2015)):

CASRN	Chemical Name	Agonist Potency	Antagonist Potency	Activity in Assay
57-63-6	17alpha-Ethinyl estradiol	Strong	Inactive	Yes
50-28-2	17beta-Estradiol	Strong	NA	Yes
56-53-1	Diethylstilbestrol (DES)	Strong	Inactive	Yes
84-16-2	meso-Hexestrol	Strong	NA	Yes
57-91-0	17alpha-Estradiol	Moderate	NA	Yes
140-66-9	4-tert-Octylphenol	Moderate	NA	Yes
53-16-7	Estrone	Moderate	NA	Yes
599-64-4	4-Cumylphenol	Weak	NA	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Inactive	Yes
80-05-7	Bisphenol A	Weak	Inactive	Yes
77-40-7	Bisphenol B	Weak	NA	Yes
486-66-8	Daidzein	Weak	NA	Yes
446-72-0	Genistein	Weak	Inactive	Yes
143-50-0	Kepone	Weak	Inactive	No
789-02-6	o,p'-DDT	Weak	NA	Yes
58-18-4	17alpha-Methyltestosterone	Very Weak	NA	Yes
520-36-5	Apigenin	Very Weak	Inactive	Yes
85-68-7	Butylbenzyl phthalate	Very Weak	Inactive	Yes

480-40-0	Chrysin	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	Inactive	No
117-81-7	Diethylhexyl phthalate	Very Weak	Inactive	No
84-74-2	Di-n-butyl phthalate	Very Weak	Inactive	No
120-47-8	Ethylparaben	Very Weak	NA	Yes
60168-88-9	Fenarimol	Very Weak	NA	No
520-18-3	Kaempferol	Very Weak	NA	Yes
72-43-5	Methoxychlor	Very Weak	NA	Yes
72-55-9	p,p'-DDE	Very Weak	Inactive	No
104-40-5	p-n-Nonylphenol	Very Weak	NA	Yes
1912-24-9	Atrazine	Inactive	NA	No
50-22-6	Corticosterone	Inactive	NA	Yes
66-81-9	Cycloheximide	Inactive	NA	No
13311-84-7	Flutamide	Inactive	NA	Yes
52-86-8	Haloperidol	Inactive	NA	No
52806-53-8	Hydroxyflutamide	Inactive	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	Yes
330-55-2	Linuron	Inactive	NA	No
57-30-7	Phenobarbital Sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	Yes
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	Yes
68392-35-8	4-Hydroxytamoxifen (E/Z)	NA	Active	Yes
82640-04-8	Raloxifene	NA	Active	Yes
10540-29-1	Tamoxifen	NA	Active	Yes
54965-24-1	Tamoxifen citrate	NA	Active	Yes
57-83-0	Progesterone	NA	Inactive	Yes

Agonist Activity	ToxCast Active	ToxCast Inactive
Active	21	7
Inactive	6	6

Antagonist Activity	ToxCast Active	ToxCast Inactive
Active	4	0
Inactive	8	6

Agonist Sensitivity = 75%

Agonist Specificity = 50%

Balanced Accuracy = 62.5%

Antagonist Sensitivity = 100%

Antagonist Specificity = 42.9%

Balanced Accuracy = 71.4%

Overall balanced accuracy for assay = 62.1%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [16].

Assay Documentation

References

[1] Russo, J. and I. H. Russo (2006). J Ster Biochem Mol Biol 102(1): 89-96. (PMID: 17113977)

[2] Yager, J. D. and N. E. Davidson (2006). New Engl J Med 354(3): 270-282.

[3] Keydar, I., et al. (1979). Eur J Cancer 15(5): 659-670. (PMID: 228940)

[4] Horwitz, K. B., et al. (1978). Cancer Res 38(8): 2434-2437. (PMID: 667841)

[5] Spink, D. C., et al. (1998). Carcinogenesis 19(2): 291-298. (PMID: 9498279)

[6] Spink, D. C., et al. (2002). Drug Metab Disposition 30(3): 262-269. (PMID: 11854143)

[7] Angus, W. G., et al. (1999). Carcinogenesis 20(6): 947-955. (PMID: 10357772)

[8] MacPherson, L. and J. Matthews (2010). Cancer Lett 299(2): 119-129. (PMID: 20846786)

[9] Hevir, N., et al. (2011). Chem-Biol Interact 191(1): 206-216. (PMID: 21182832)

[10] Lo, R., et al. (2010). Biochimica et Biophysica Acta 1799(5): 469-479. (PMID: 20079471)

[11] Nagaoka, R., et al. (2006). Endocrine 30(3): 261-268. (PMID: 17526937)

[12] Mitra, R., et al. (2011). J Biol Chem 286(20): 17543-17559. (PMID: 21402692)

[13] Harrington, W. R., et al. (2006). Endocrinology 147(8): 3843-3850. (PMID: 16690804)

[14] Miki, Y., et al. (2006). Cancer Res 66(1): 535-542. (PMID: 16397270)

[15] Judson, R. S., et al. (2015). Toxicol Sci 148(1): 137-154. (PMID)

[16] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

15 November 2016

Author of Revisions:

EPA NCCT

Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

Assay Endpoint ID: 115

ATG_AR_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Androgen Receptor Activation Assay

Assay Descriptions

Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL^{TM}) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 (Aden et al. 1979), which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 (Westerink and Schoonen 2007a) with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions (Hewitt and Hewitt 2004); some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes (Guo et al. 2010). Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 (Hart et al. 2010, Walle et al. 2000, Westerink and Schoonen 2007b) and UGTs (1A1, 1A6 and 2B7) (Hart et al. 2010). In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein (Boehme et al. 2010) and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g.,

ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) (Adachi et al. 2007).

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95 °C, 20 s at 68 °C and 10 min at 72 °C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37 °C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

Attagene second generation of TRANS-FACTORIAL assays were conducted with an expanded NR platform, which covers all 48 human NRs.

Assay Component Descriptions

Assay Objectives:

The Attagene TRANS Androgen Receptor assay measures changes in human androgen-receptor (AR) activation using the mammalian one-hybrid assay format, which monitored transcriptional activity of

the AR ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection. The presence of AR agonists alters the transactivation function of Gal4-AR RTU and modulates reporter transcription. Following 24-hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the RTU sequences followed by quantitation by capillary electrophoresis. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures used). Following MTC determination, concentration-response assays where conducted by exposing a single replicate to a 6-8 point concentration series, starting at the MTC and followed by 3-fold serial dilutions. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls.

ATG_AR_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_AR_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-AR proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an AR-specific reporter sequence. Chemical-AR activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting androgen signaling pathways. The androgen receptor mediates gene expression in response to androgenic exposures, and modulates the activity for a wide variety of physiological processes. The ATG_AR_TRANS assay used a hepatoma cell-based platform to monitor androgen receptor transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for endocrine disrupting activity via interactions with androgen receptor.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:
Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)
Assay Quality Statistics:

Neutral control well median response value, by plate:	1.1575
Neutral control median absolute deviation, by plate:	0.2646
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.23

Assay Endpoint Descriptions
Data Interpretation
Biological Response:
Androgen receptor agonism - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to ligand binding of human androgen receptor as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.
Analytical Elements:
ATG_AR_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.176
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.881

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:
The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

Assay Documentation

References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)
[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)
[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)
[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)
[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)
[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)
[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)
[9] Adachi, T., et al. (2007). Journal of Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EAGMST, Extended Advisory Group on Molecular Screening and Toxicogenomics
EDC, Endocrine disrupting chemicals
GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit
MIE, Molecular Initiating Event
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:
Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation
Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;
Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

Supporting Information (existing annotations):

ATG_ERa_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Estrogen Receptor Alpha Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by chimeric GAL4-NR transcription factors utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by the yeast GAL4 DNA-binding domain. This family of Attagene assays employ a recently developed profiling technology (Factorial™) which consists of multiple RTU construct sequences that are identical with the exception of processing tag sequences assigned to each TF which create a unique restriction enzyme cleavage site for individual RTUs, and allow for specific determination of NR activity. A specific MRTU paired with a specific chimeric GAL4-NR protein are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19) and, 24 hr after transfection, transfected cells for all 25 nuclear receptors and MRTU's are mixed and plated. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by quantitating transcribed reporter RNA through cDNA synthesis, fluorescent labeling, and restriction enzyme digestion to yield specific reporter products. This trans-format Factorial™ assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by a single reaction creating highly homogeneous detection conditions.

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates

genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95 °C, 20 s at 68 °C and 10 min at 72 °C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37 °C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The estrogen receptor used in this assay is a partial receptor consisting of the ligand-binding domain and hinge region and may not represent the physiological form of the receptor. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.2. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

Attagene second generation of TRANS-FACTORIAL assays were conducted with an expanded NR platform, which covers all 48 human NRs.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS Estrogen Receptor alpha assay measures changes in human estrogen-receptor alpha (hERα) activation using the mammalian one-hybrid assay format, which monitored transcriptional activation using hERα ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection, and one replicate of each compound was screened at eight concentrations of each test compound. The presence of agonists/antagonists of ERα alters the transactivation function of Gal4-NR and modulates reporter transcription. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_ERa_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_ERa_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-ERα proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an ERα-specific reporter sequence. Chemical-ERα activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The ATG_ERa_TRANS assay used a hepatoma cell-based platform to monitor estrogen receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenoestrogenic activity. This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates (AOP under development), and there is some evidence that estrogen

receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (AOPs under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.77
Neutral control median absolute deviation, by plate:	0.50
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
CV (median across all plates):	0.28

(no positive control used for this assay)

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen receptor agonism - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to ligand binding of human estrogen receptor α as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_ERa_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a signal baseline. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_LUC_BG1_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 8
Target (nominal) number of replicates: 1
Standard minimum concentration tested: 0.09 µM
Standard maximum concentration tested: 200 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.23
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 1.13

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	No
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	Yes
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	Yes

104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	Yes
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No

57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	24	13
Inactive	17	5

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	34	8
Inactive	7	10

In Vitro Sensitivity = 64.9%
In Vitro Specificity = 22.7%
Balanced Accuracy = 43.8%

In Vivo Sensitivity = 81.0%
In Vivo Specificity = 58.8%
Balanced Accuracy = 69.9%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)
[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)
[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)
[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)
[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)
[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)
[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)
[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
ERE, Estrogen Response Element
E2, Estradiol
GST, Glutathione S-Transferase
MIE, Molecular Initiating Event
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

15 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

ATG_ERb_TRANS2_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Estrogen Receptor Beta Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The second series of trans-acting Attagene assays (TRANS2) track changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (Factorial™) which consists of multiple RTU construct sequences that are identical with the exception of processing tag sequences assigned to each TF which create a unique restriction enzyme cleavage site for individual RTUs, and allow for specific determination of NR activity. A specific MRTU paired with a specific chimeric GAL4-NR protein are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19) and, 24 hr after transfection, transfected cells for all 25 nuclear receptors and MRTU's are mixed and plated. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by quantitating transcribed reporter RNA through cDNA synthesis, fluorescent labeling, and restriction enzyme digestion to yield specific reporter products. This trans-format Factorial™ assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 23 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by a single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2 to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells

also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95 °C, 20 s at 68 °C and 10 min at 72 °C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37 °C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The estrogen receptor used in this assay is a partial receptor consisting of the ligand-binding domain and hinge region and may not represent the physiological form of the receptor. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None Reported

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS2 Estrogen Receptor beta assay measures changes in estrogen-receptor beta (ERβ) activation using the mammalian one-hybrid assay format, which monitored transcriptional activation using ERβ ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection, and one replicate of each compound was screened at eight concentrations of each test compound. the presence of agonists/antagonists of ERβ alters the transactivation function of Gal4-NR and modulates the reporter signal. Following 24-hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_ERb_TRANS2 series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_ERb_TRANS2 is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-ERβ proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an ERβ-specific reporter sequence. Chemical-ERβ activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The ATG_ERa_TRANS assay used a hepatoma cell-based platform to monitor estrogen receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenoestrogenic activity. This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates (AOP under development), and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (AOPs under development). Chemical-activity profiles derived from this assay can inform

prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.6625
Neutral control median absolute deviation, by plate:	0.09118
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen receptor agonism - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to ligand binding of human estrogen receptor β as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_ERb_TRANS2_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a signal baseline. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor beta activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test

chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_LUC_BG1_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.04 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2125
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.063

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and

ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
ERE, Estrogen Response Element
E2, Estradiol
GST, Glutathione S-Transferase
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

ATG_ERE_CIS_up

Assay Title: Attagene CIS-FACTORIAL HepG2 Estrogen Response Element Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene CIS assays track changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual transcription factor response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of cis-regulating element (promoter) binding by RTUs. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and the CIS- format assay measures changes in RTU expression resulting from TF binding to response element DNA-binding sites. Response to 24-hour incubation of test chemicals with cells in a 24-well plate is monitored by examining fluorescent activity produced by transcribed mRNA. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions in a single-replicate 8-point chemical concentration series.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2 to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These

transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95 °C, 20 s at 68 °C and 10 min at 72 °C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37 °C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

Due to low expression levels of estrogen receptor in HepG2 cells, a full-length, human estrogen receptor α cDNA was co-transfected in to the cells together with the MRTUs. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None Reported

2. Assay Component Descriptions

Assay Objectives:

The Attagene CIS estrogen response element assay measures changes in the mRNA production controlled by a cis-acting element (promoter). Multiple RTU constructs are transfected into the human liver hepatoma cell line HepG2, and the cis- format assay measures changes in RTU expression resulting from endogenous TF binding, i.e. estrogen receptor α or β, to estrogen response element (ERE) DNA-binding sites following 24 hour exposures to the ToxCast chemical library. TF activity was reported via cDNA synthesis and RT-PCR of the RTU sequences followed by

quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_CIS series assays consist of multiplexed (FACTORIAL™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP) cDNA and each tagged with *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurable with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. The ATG_ERE_CIS describes MRTU FACTORIAL™ assays reporting activity in endogenous cis-regulatory estrogen response element (ERE) constructs (which are responsive to endogenous human estrogen receptor α and β).

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The ATG_ERE_CIS assay used a hepatoma cell-based platform to monitor estrogen receptor transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenoestrogenic activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds which potentially affect endocrine systems in exposed populations by interacting with estrogen receptor mediated signaling pathways. There is evidence that estrogen receptor activation in early life is a molecular initiating event (MIE) in a developing Adverse Outcome Pathways (AOP) leading to endometrial carcinoma in the mouse (currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER interference in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.88
Neutral control median absolute deviation, by plate:	0.19
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using positive control wells):	NA

Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
CV (median across all plates):	0.21

(no positive control used for this assay)

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity; increased production of mRNA transcripts production in response to active transcription following TF interaction with ERE promoter sequences as measured by RT_PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_ERE_CIS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor transactivation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_LUC_BG1_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 8
Target (nominal) number of replicates: 1
Standard minimum concentration tested: 0.09 µM
Standard maximum concentration tested: 200 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.10
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 0.50

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	Yes
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	Yes
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes

480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	Yes
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	Yes
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	28	9
Inactive	17	5

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	36	6
Inactive	9	8

In Vitro Sensitivity = 75.7%

In Vitro Specificity = 22.7%
Balanced Accuracy = 49.2%

In Vivo Sensitivity = 85.7%
In Vivo Specificity = 47.1%
Balanced Accuracy = 66.4%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)
[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)
[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)
[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)
[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)
[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)
[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)
[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)
[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
ERE, Estrogen Response Element

E2, Estradiol
GST, Glutathione S-Transferase
MIE, Molecular Initiating Event
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

ATG_ERRa_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Estrogen-Related Receptor Alpha Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (Factorial™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format Factorial™ assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells

also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95 °C, 20 s at 68 °C and 10 min at 72 °C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37 °C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

Due to low expression levels of estrogen receptor in HepG2 cells, a full-length, human estrogen receptor α cDNA was co-transfected in to the cells together with the MRTUs. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene Estrogen-Related Receptor alpha TRANS assay measures changes in an ERRα activation using the mammalian one-hybrid assay format. A chimeric yeast GAL4 DNA binding domain-human ERRα ligand binding domain protein regulates expression of a specific RTU through the UAS promoter that binds the GAL4-DNA binding domain. The presence of agonists/antagonists of ERRα alters the transactivation function of Gal4-NR and modulates reporter transcription. ERRs are orphaned nuclear receptors with significant homology to the estrogen receptor family; they share target genes, co-regulators and promoters, and have an almost identical DNA binding domain and bind similarly to EREs in the nucleus, but are responsive to different ligands. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC50) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_ERRa_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_ERRa_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-NR proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an ERRα-specific reporter sequence. Chemical-ERRα activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. Estrogenic DNA binding mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The ATG_ERRa_TRANS assay used a hepatoma cell-based platform to monitor estrogen-related receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenoestrogenic activity.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.45
Neutral control median absolute deviation, by plate:	0.40
Positive control well median response value, by plate:	NA

Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity; increased production of mRNA transcripts in response to ligand binding to estrogen-related receptor α as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_ERRa_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a signal baseline. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. ERR activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_ERRa_TRANS_up
ATG_ERRb_TRANS2_up
ATG_ERRg_TRANS_up

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM

Standard maximum concentration tested:	200.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.24
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor

ERE, Estrogen Response Element
E2, Estradiol
GST, Glutathione S-Transferase
MIE, Molecular Initiating Event
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

ATG_ERRb_TRANS2_up

Assay Title: Attagene TRANS2-FACTORIAL HepG2 Human Estrogen-Related Receptor Beta Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The second series of trans-acting Attagene assays (TRANS2) track changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against all 48 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2 to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells

also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

Due to low expression levels of estrogen receptor in HepG2 cells, a full-length, human estrogen receptor α cDNA was co-transfected in to the cells together with the MRTUs. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene Estrogen-Related Receptor beta TRANS2 assay measures changes in an ERRβ activation using the mammalian one-hybrid assay format. A chimeric yeast GAL4 DNA binding domain-human ERRβ ligand binding domain protein regulates expression of a specific RTU through the UAS promoter that binds the GAL4-DNA binding domain. The presence of agonists/antagonists of ERRβ alters the transactivation function of Gal4-NR and modulates reporter transcription. ERRs are orphaned nuclear receptors with significant homology to the estrogen receptor family; they share target genes, co-regulators and promoters, and have an almost identical DNA binding domain and bind similarly to EREs in the nucleus, but are responsive to different ligands. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_ERRβ_TRANS2 series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_ERRβ_TRANS2 is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-NR proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an ERRβ-specific reporter sequence. Chemical-ERRβ activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. Estrogenic DNA-binding mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The ATG_ERRb_TRANS2 assay used a hepatoma cell-based platform to monitor estrogen-related receptor beta transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenoestrogenic activity.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.72
Neutral control median absolute deviation, by plate:	0.478
Positive control well median response value, by plate:	NA

Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.28

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity; increased production of mRNA transcripts in response to ligand binding of estrogen-related receptor β as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_ERRβ_TRANS2_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a signal baseline. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. ERR beta activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_ERRa_TRANS_up
ATG_ERRb_TRANS2_up
ATG_ERRg_TRANS_up

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.04 µM

Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.457
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	2.284

Reference Chemicals / Predictive Capacity:

Rationale for selection of chemical library:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). Journal of Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor

ERE, Estrogen Response Element
E2, Estradiol
GST, Glutathione S-Transferase
MIE, Molecular Initiating Event
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

ATG_ERRg_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Estrogen-Related Receptor Gamma Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (Factorial™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format Factorial™ assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2 to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells

also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

Due to low expression levels of estrogen receptor in HepG2 cells, a full-length, human estrogen receptor α cDNA was co-transfected in to the cells together with the MRTUs. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene Estrogen-Related Receptor gamma TRANS assay measures changes in an ERRγ activation using the mammalian one-hybrid assay format. A chimeric yeast GAL4 DNA binding domain-human ERRγ ligand binding domain protein regulates expression of a specific RTU through the UAS promoter that binds the GAL4-DNA binding domain. The presence of agonists/antagonists of ERRγ alters the transactivation function of Gal4-NR and modulates reporter transcription. ERRs are orphaned nuclear receptors with significant homology to the estrogen receptor family; they share target genes, co-regulators and promoters, and have an almost identical DNA binding domain and bind similarly to EREs in the nucleus, but are responsive to different ligands. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_ERRg_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_ERRg_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-NR proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an ERRγ-specific reporter sequence. Chemical-ERRγ activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. Estrogenic DNA-binding mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The ATG_ERRg_TRANS assay used a hepatoma cell-based platform to monitor estrogen-related receptor gamma transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenoestrogenic activity.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.31
Neutral control median absolute deviation, by plate:	1.56
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA

Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.47

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity; increased production of mRNA transcripts in response to ligand binding of estrogen-related receptor gamma as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_ERRβ_TRANS2_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a signal baseline. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. ERR gamma activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_ERRa_TRANS_up
ATG_ERRb_TRANS2_up
ATG_ERRg_TRANS_up

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2777
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.386

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). Journal of Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
ERE, Estrogen Response Element
E2, Estradiol

GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

ATG_FXR_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Farnesoid X Receptor Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export

pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo (dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote FXR mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

Attagene's second generation of TRANS-FACTORIAL assays were conducted with an expanded NR platform, which covers all 48 human NRs.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS Farnesoid X Receptor alpha assay measures changes in FXR activation using the mammalian one-hybrid assay format, which monitored transcriptional activity of the FXR

ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection. The presence of agonists/antagonists of FXR alters the transactivation function of Gal4-NR and modulates reporter transcription. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the RTU sequences followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_FXR_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_FXR_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-FXR proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of a FXR-specific reporter sequence. Chemical-FXR activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Farnesoid-X-receptor (FXR) is a ligand-activated nuclear whose primary function is to act as a bile acid sensor, protecting the liver from bile acid toxicity by regulating the transcription of genes involved in bile acid homeostasis [10]. Genes associated with bile acid biosynthesis and recycling (CYP7A1 and IBABP, respectively) are known targets of FXR [11]. FXR has a role in the regulation of glucose and lipid metabolic pathways. FXR is primarily expressed in the liver, kidney, intestine and adrenal cortex, and regulates the expression of target genes by binding as a heterodimer with the retinoid X receptor (RXR). The FXR/RXR heterodimer is "permissive" in that the pair becomes transcriptionally active in the presence of either an RXR-selective ligand or a FXR ligand [8]. The FXR-RXR heterodimer, when bound to DNA, can act as transcriptional activators or inhibitors. FXR is activated by bile acids and the main endogenous ligand for FXR is chenodeoxycholic acid (CDCA). FXR reduces bile acid concentration in the liver by repressing genes involved in bile acid synthesis and regulates lipid metabolism. Numerous studies have reported that FXR exerts protective function during cholestasis, diabetes, liver regeneration, and cancer. The ATG_FXR_TRANS assay used a hepatoma cell-based platform to monitor farnesoid X receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for endocrine disrupting activity via interactions with farnesoid X receptor.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system

enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.24
Neutral control median absolute deviation, by plate:	0.286
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to ligand binding of farnesoid X receptor as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_FXR_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up

NVS_NR_hFXR_Agonist
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_0480
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.169
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.846

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [12].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)
[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)
[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)
[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)
[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)
[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)
[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)
[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)
[10] Kim, I., et al. (2007). J Lipid Res 48(12): 2664-2672. (PMID: 17720959)
[11]Laudet, V. and H. Gronemeyer (2001). The Nuclear Receptor Factsbook, Gulf Professional Publishing.
[12] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
FXR, Farnesoid-X-Receptor
GST, Glutathione-S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

20 October 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

ATG_IR1_CIS_up

Assay Title: Attagene CIS-FACTORIAL HepG2 Farnesoid X Response Element IR1 Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene CIS assays track changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual transcription factor response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of cis-regulating element (promoter) binding by RTUs. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and the CIS- format assay measures changes in RTU expression resulting from TF binding to response element DNA-binding sites. Response to 24-hour incubation of test chemicals with cells in a 24-well plate is monitored by examining fluorescent activity produced by transcribed mRNA. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions in a single-replicate 8-point chemical concentration series.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2 to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These

transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

Due to low expression levels of estrogen receptor in HepG2 cells, a full-length, human estrogen receptor α cDNA was co-transfected in to the cells together with the MRTUs. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote FXR-IR1 mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene CIS IR1 FXR response element assay measures changes in the mRNA production controlled by a cis-acting element (promoter). Multiple RTU constructs are transfected into the human liver hepatoma cell line HepG2, and the cis- format assay measures changes in RTU expression resulting from endogenous TF binding, i.e. FXR ligand binging to inverted repeat sequences separated by 1 base pair (IR1) which are responsive to FXR-RXR heterodimer formation, following 24 hour exposures to the ToxCast chemical library. TF activity was reported via cDNA

synthesis and RT-PCR of the RTU sequences followed by quantitation by capillary electrophoresis. Half-maximal activity (IC50) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_CIS series assays consist of multiplexed (FACTORIAL) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP) cDNA and each tagged with HpaI restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurable with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. The ATG_IR1_CIS describes MRTU FACTORIAL assays reporting activity in endogenous cis-regulatory FXR response element (IR1) constructs.

Scientific Principles:

Farnesoid-X-receptor (FXR) is a ligand-activated nuclear whose primary function is to act as a bile acid sensor, protecting the liver from bile acid toxicity by regulating the transcription of genes involved in bile acid homeostasis [10]. Genes associated with bile acid biosynthesis and recycling (CYP7A1 and IBABP, respectively) are known targets of FXR [11]. FXR has a role in the regulation of glucose and lipid metabolic pathways. FXR is primarily expressed in the liver, kidney, intestine and adrenal cortex, and regulates the expression of target genes by binding as a heterodimer with the retinoid X receptor (RXR). The FXR/RXR heterodimer is "permissive" in that the pair becomes transcriptionally active in the presence of either an RXR-selective ligand or a FXR ligand [8]. The FXR-RXR heterodimer, when bound to DNA, can act as transcriptional activators or inhibitors. FXR is activated by bile acids and the main endogenous ligand for FXR is chenodeoxycholic acid (CDCA). FXR reduces bile acid concentration in the liver by repressing genes involved in bile acid synthesis and regulates lipid metabolism. Numerous studies have reported that FXR exerts protective function during cholestasis, diabetes, liver regeneration, and cancer. The ATG_FXR_TRANS assay used a hepatoma cell-based platform to monitor farnesoid X receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for endocrine disrupting activity via interactions with farnesoid X receptor.

Method Development Reference:

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.633
Neutral control median absolute deviation, by plate:	0.156
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	NA
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA

Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.25

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to DNA binding of farnesoid X receptor response element IR1 as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_IR1_CIS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR transactivation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_FXR_TRANS_up
NVS_NR_hFXR_Agonist
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_0480
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0976
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.488

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [12].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). Journal of Experimental Therapeutics and Oncology 6(4): 335-348. (PMID: 18038766)

[10] Kim, I., et al. (2007). J Lipid Res 48(12): 2664-2672. (PMID: 17720959)

Laudet, V. and H. Gronemeyer (2001). The Nuclear Receptor Factsbook, Gulf Professional Publishing.

[12] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion

ATCC, American Tissue Culture Collection

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals
FXR, Farnesoid-X-Receptor
GST, Glutathione-S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

6 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

ATG_PPARa_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Peroxisome Proliferator-activated Receptor Alpha (PPARα) Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export

pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95 °C, 20 s at 68 °C and 10 min at 72 °C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37 °C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote PPAR mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

Attagene second generation of TRANS-FACTORIAL assays were conducted with an expanded NR platform, which covers all 48 human NRs.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS Peroxisome Proliferator-Receptor (PPAR) α assay measures changes in human PPAR alpha activation using the mammalian one-hybrid assay format, which monitored transcriptional activity of the AR ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection. The presence of AR agonists alters the transactivation function of Gal4-AR RTU and modulates reporter transcription. Following 24-hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the RTU sequences followed by quantitation by capillary electrophoresis. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures used). Following MTC determination, concentration-response assays where conducted by exposing a single replicate to a 6-8 point concentration series, starting at the MTC and followed by 3-fold serial dilutions. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls.

ATG_AR_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_AR_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-AR proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of an AR-specific reporter sequence. Chemical-AR activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Peroxisome proliferator-activated receptor alpha (PPARα) is a ligand-activated nuclear receptor which regulates the expression of genes involved in fatty acid-oxidation and is a major regulator of energy homeostasis. PPARα plays a crucial role in the regulation of proteins involved in fatty acid transport and hepatic uptake, is expressed predominantly in metabolically active tissues, including liver, kidney, skeletal muscle, and brown fat and is a target for hyperlipidemia drugs, fatty acids (and their derivative eicosanoids) and xenobiotics. The ATG_PPARa_TRANS assay used a hepatoma cell-based platform to monitor PPARα transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenobiotic PPARα ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARa) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that PPARa activation in utero is the molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to impaired fertility in males (AOP Under EAGMST Review), and there is some evidence that PPARa activation is the MIE for a putative pathways leading to hepatocellular adenomas and carcinomas and may

be involved in increased pancreatic acinar tumors (in mouse and rat models) (AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPARa activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.455
Neutral control median absolute deviation, by plate:	1.334
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.39

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPARα agonism; nuclear steroid hormone receptor initiated production of mRNA transcripts in response to ligand binding of peroxisome proliferator-activated receptor alpha as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_PPARa_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR alpha activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss

functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publically available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
NVS_NR_hPPARa

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.24
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)
[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)
[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)
[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)
[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)
[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)
[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)
[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
PPAR, Peroxisome Proliferator-Activated Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 September 2016

Date of Revisions:

21 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 133

ATG_PPARd_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Peroxisome Proliferator-activated Receptor Delta (PPARd) Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24 hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export

pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

Factorial™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

Attagene second generation of TRANS-FACTORIAL assays were conducted with an expanded NR platform, which covers all 48 human NRs.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS peroxisome proliferator-activated receptor delta (PPARδ) assay measures changes in human PPARδ activation using the mammalian one-hybrid assay format, which monitored transcriptional activation using PPARδ ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection, and one replicate of each compound was screened at eight concentrations of each test compound. The presence of agonists/antagonists of PPARδ alters the transactivation function of Gal4-NR and modulates reporter transcription. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_PPARd_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_PPARd_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-ERα proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of a PPARδ-specific reporter sequence. Chemical-PPARδ activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Peroxisome proliferator-activated receptor delta is a ligand-activated nuclear receptor which is expressed ubiquitously and may have a role in regulating the differentiation of adipocytes, in keratinocyte differentiation and in the regulation of cholesterol and lipid metabolism [10, 11]. The ATG_PPARd_TRANS assay used a hepatoma cell-based platform to monitor PPARδ transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenobiotic PPARδ ligand-binding activity.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.781
Neutral control median absolute deviation, by plate:	0.337
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA

Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.43

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPARd agonism; nuclear steroid hormone receptor initiated production of mRNA transcripts in response to ligand binding of peroxisome proliferator-activated receptor delta as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_PPARd_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR delta receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
Tox21_PPARd_BLA_agonist_ratio
Tox21_PPARd_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.225
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.123

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [12].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). Journal of Experimental Therapeutics and Oncology 6(4): 335-348. (PMID: 18038766)

[10] Schmuth, M., et al. (2004). Journal of Investigative Dermatology 122(4): 971-983.

[11] Seimandi, M., et al. (2005). Anal Biochem 344(1): 8-15. (PMID: 16038868)

[12] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
GST, Glutathione S-Transferase

MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
PPAR, Peroxisome Proliferator-Activated Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 September 2016

Date of Revisions:

21 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

ATG_PPARg_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Peroxisome Proliferator-activated Receptor Gamma (PPARg) Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export

pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

Factorial™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

Attagene second generation of TRANS-FACTORIAL assays were conducted with an expanded NR platform, which covers all 48 human NRs.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS peroxisome proliferator-activated receptor gamma (PPARγ) assay measures changes in human PPARγ activation using the mammalian one-hybrid assay format, which monitored transcriptional activation using PPARγ ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection, and one replicate of each compound was screened at eight concentrations of each test compound. The presence of agonists/antagonists of PPARγ alters the transactivation function of Gal4-NR and modulates reporter transcription. Following 24-hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_PPARg_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_PPARg_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-ERα proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of a PPARγ-specific reporter sequence. Chemical-PPARγ activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor which regulates the expression of genes involved in fatty acid-oxidation and is a major regulator of energy homeostasis. PPARγ is primarily expressed in adipose tissue, macrophages and in the colon where it controls adipocyte differentiation, lipid storage and inflammatory responses. PPARγ agonists, the thiazolidinediones (TZDs), improve insulin sensitivity, lower glucose levels, and lower plasma triglycerides and free fatty acid (FFA) levels by enhancing their uptake into adipocytes. The ATG_PPARg_TRANS assay used a hepatoma cell-based platform to monitor PPARγ transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for xenobiotic PPARγ ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARg) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is some evidence to support a putative AOP linking PPAR gamma receptor activation with increased occurrence of sarcomas in rats, mice, and hamsters (AOP currently under development). Chemical-activity profiles derived from this assay

can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPAR activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.66
Neutral control median absolute deviation, by plate:	1.08
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.41

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPARg agonism; nuclear steroid hormone receptor initiated production of mRNA transcripts in response to ligand binding of peroxisome proliferator-activated receptor gamma as measured by RT-PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_PPARg_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR gamma activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical.

Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
NVS_NR_hPPARg
OT_PPARg_PPARgSRC1_0480
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_Agonist_ratio
Tox21_PPARg_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.235
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)
[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)
[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)
[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)
[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)
[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)
[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)
[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
PPAR, Peroxisome Proliferator-Activated Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 September 2016

Date of Revisions:

21 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 102

ATG_PPRE_CIS_up

Assay Title: Attagene CIS-FACTORIAL HepG2 Peroxisome Proliferator-activated Response Element Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene CIS assays track changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual transcription factor response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL^{TM}) which consists of cis-regulating element (promoter) binding by RTUs. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and the CIS- format assay measures changes in RTU expression resulting from TF binding to response element DNA-binding sites. Response to 24-hour incubation of test chemicals with cells in a 24-well plate is monitored by examining fluorescent activity produced by transcribed mRNA. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions in a single-replicate 8-point chemical concentration series.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo(dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

FACTORIAL™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

Due to low expression levels of estrogen receptor in HepG2 cells, a full-length, human estrogen receptor α cDNA was co-transfected in to the cells together with the MRTUs. The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene CIS peroxisome proliferator-activated response element assay measures changes in the mRNA production controlled by a cis-acting element (promoter) (PPRE). Multiple RTU constructs are transfected into the human liver hepatoma cell line HepG2, and the cis- format assay

measures changes in RTU expression resulting from endogenous TF binding, i.e. PPAR activation, to response element (PPRE) DNA-binding sites following 24 hour exposures to the ToxCast chemical library. TF activity was reported via cDNA synthesis and RT-PCR of the RTU sequences followed by quantitation by capillary electrophoresis. Modulation of PPRE activity was monitored using a positive control of Rosiglitazone. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_CIS series assays consist of multiplexed (FACTORIAL™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP) cDNA and each tagged with *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurable with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. The ATG_PPRE_CIS describes MRTU FACTORIAL™ assays reporting activity in endogenous cis-regulatory peroxisome proliferator-activated response element (PPRE) constructs.

Scientific Principles:

Peroxisome proliferator-activated receptors are transcription factors belonging to the family of ligand-inducible nuclear receptors; they regulate the expression of genes involved in many cellular and metabolic processes, and are involved in fatty acid-oxidation, energy homeostasis and function in epidermal differentiation and repair. They regulate glucose, lipid, and cholesterol metabolism in response to fatty acids and their derivatives, eicosanoids, and are targets for drugs used in the treatment of hyperlipidemia and diabetes. There are three distinct subtypes; PPAR alpha, PPAR delta (also called PPAR beta) and PPAR gamma, each of which show distinct tissue distribution and ligand preference. All these subtypes heterodimerize with Retinoid X receptor (RXR) and these heterodimers regulate transcription of various genes. PPARα is expressed predominantly in metabolically active tissues, including liver, kidney, skeletal muscle, and brown fat, and its ligands include fatty acids, hypolipidemic drugs, and xenobiotics, where PPARβ/δ is the target for antidiabetic agents of the thiazolidinedione class, and is expressed ubiquitously, and may be involved in the regulation of cholesterol and lipid metabolism. PPARγ is highly expressed in adipocytes, is involved in control of lipid storage and is thought to be important in the induction of adipogenesis. The ATG_PPRE_CIS assay used a hepatoma cell-based platform to monitor gene expression resulting from PPAR response element activation which is regulated by PPAR receptor ligand-binding activity and this assay is designed to help identify environmental compounds with a capacity for xenobiotic PPAR ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARa) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that PPARa activation in utero is the molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to impaired fertility in males (AOP Under EAGMST Review), and there is some evidence that PPARa activation

is the MIE for a putative pathways leading to hepatocellular adenomas and carcinomas and may be involved in increased pancreatic acinar tumors (both AOPs based on evidence in mouse and rat models) (AOPs currently under development). There is also a putative AOP linking PPAR gamma receptor activation with increased occurrence of sarcomas in rats, mice, and hamsters (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPAR activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.26
Neutral control median absolute deviation, by plate:	0.627
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.5

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity – mRNA transcript production in response to active transcription following TF interaction with PPRE promoter sequences as measured by RT_PCR and capillary electrophoretic detection of fluorescently labeled mRNA.

Analytical Elements:

ATG_PPRE_CIS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Negative and zero values are removed before analysis and raw values are log transformed. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR receptor transactivation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise

band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPARa_TRANS_up
ATG_PPARd_TRANS_up
ATG_PPARg_TRANS_up
NVS_NR_hPPARa
NVS_NR_hPPARg
OT_PPARg_PPARgSRC1_0480
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_Agonist_ratio
Tox21_PPARd_BLA_agonist_ratio
Tox21_PPARd_BLA_antagonist_ratio
Tox21_PPARg_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.180
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.900

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical

inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
PPAR, Peroxisome Proliferator-Activated Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
TF, Transcription Factor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 September 2016

Date of Revisions:

22 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 143

ATG_THRa1_TRANS_up

Assay Title: Attagene TRANS-FACTORIAL HepG2 Human Thyroid Receptor Alpha Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Attagene TRANS assay tracks changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL™) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (variant HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against 25 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo (dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

Factorial™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote thyroid receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS Thyroid Receptor alpha assay measures changes in thyroid-receptor alpha (TRα) activation using the mammalian one-hybrid assay format, which monitored transcriptional activity of the TRα ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection. The presence of

agonists/antagonists of TRα alters the transactivation function of Gal4-NR and modulates reporter transcription. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the RTU sequences followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_TRa_TRANS series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_THRa1_TRANS is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-TRα proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of a TRα-specific reporter sequence. Chemical-TRα activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting thyroid hormone signaling pathways. The thyroid receptor mediates gene expression in response to thyroid hormones T3 and T4, and modulates the activity for a wide variety of physiological processes. The ATG_THRa1_TRANS assay used a hepatoma cell-based platform to monitor thyroid receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for endocrine disrupting activity via interactions with thyroid receptor.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9215
Neutral control median absolute deviation, by plate:	0.2454
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA

Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to ligand binding of thyroid receptor α.

Analytical Elements:

ATG_THRa1_TRANS_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Each chemical is run as a single replicate in a 6-point concentration series, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Thyroid receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRb_TRANS2_up
LTEA_HepaRG_THRSP_dn
LTEA_HepaRG_THRSP_up
NVS_NR_hTRa_Antagonist
Tox21_TR_LUC_GH3_Agonist
Tox21_TR_LUC_GH3_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.09 µM
Standard maximum concentration tested:	200.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.225
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.127

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit
NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
T3, Triiodothyronine
T4, Thyroxine
TF, Transcription Factor

TH, Thyroid Hormone
TR, Thyroid Receptor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

20 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

ATG_THRb_TRANS2_up

Assay Title: Attagene TRANS2-FACTORIAL HepG2 Human Thyroid Receptor Beta Activation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The second series of trans-acting Attagene assays (TRANS2) track changes in transcription factor (TF) activity in response to chemical perturbations by utilizing a library of multiple reporter transcription unit (MRTU) constructs regulated by individual TF response elements. This family of Attagene assays employ a recently developed profiling technology (FACTORIAL) which consists of trans-acting TF DNA binding sites. The multiple RTU construct sequences are identical with the exception of processing tag sequences assigned to each TF which create a unique cleavage site for individual RTUs, and allow for precise determination of NR activity. The MRTUs are transfected into an in-house clone of human liver hepatoma cell line HepG2 (HG19), and each RTU expresses a chimeric GAL4-NR protein that regulates transcription of a reporter sequence. Nuclear receptor binding by exogenous compounds alters the transactivation function of Gal4-NR and modulates reporter transcription. The chemical-NR activity is monitored by examining fluorescent activity produced by transcribed mRNA. This trans-format FACTORIAL assay was used to evaluate agonistic/antagonistic properties of the ToxCast chemical library against all 48 human nuclear receptors following 24-hour incubation with cells in a 24-well plate in a single-replicate 8-point concentration series. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions.

1.2. Assay Definition

Assay Throughput:

Transfected HepG2 cells are aliquoted into 24-well microtiter plates and incubated with test compounds for 24 hours prior to PCR detection of total RNA transcription using capillary electrophoresis.

Experimental System:

The HepG2 cell line is a permanent cell culture isolated from the liver tumor lobectomy of a 15-yr-old Caucasian male from Argentina in 1975 [1], which has been cloned and transfected with a library of multiple reporter transcription units (– see section 2, Assay Component Descriptions, for detailed definition of MRTUs).

Xenobiotic Biotransformation Potential:

The HepG2 cells used in this assay are variant HG19, a cell line selected for enhanced xenobiotic metabolism. These cells express 2- to 13 times more cytochrome P450 activity than parental HepG2 (Attagene, personal communication). The parental HepG2 cell line has been shown by others to retain the potential for Phase I and Phase II metabolic responses to xenobiotics, e.g., expression of CYP1A1/2, 2A6, 2B6, 2C8/9, 2C19, 2D6/3A, 2E1, and 3A4/5 [2] with CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A activities reported at levels similar to human hepatocytes although variable depending on source and culture conditions [3]; some enzymes (e.g., CYP2W1) have even been observed at higher rates than in primary hepatocytes [4]. Phase II enzyme activities identified in HepG2 cells include SULTS (1A1, 1A2, 1E1 and 2A1), GSTs (mGST-1, GST µ1), NAT1, EPHX1 [5-7] and UGTs (1A1, 1A6 and 2B7) [7]. In addition, HepG2 cells can potentially express xenobiotic regulation activities via functionally active p53 protein [8] and Nrf2, a transcription factor which regulates genes containing antioxidant response element (ARE) sequences in their promoters; HepG2 cells also possess the capacity to express a number of ATP-binding cassette (ABC) xenobiotic export

pumps (e.g., ABCC1, C2, C3 and G2 – membrane-bound proteins also regulated in part by Nrf2 TF DNA-binding) [9].

Basic Procedure:

Human liver HepG2 cells are transiently transfected with multiple reporter transcription units (MRTUs) in 6-well plates using FuGene 6 reagent (Roche; 3 ml FuGene/1 mg DNA). These transfected cells are aliquoted into wells of a 24-well plate, exposed to the chemical library of environmental compounds for 24 hours, and total RNA was isolated using TriZol reagent (Invitrogen). RNA was reverse-transcribed using oligo (dT) primer and Mo-MLV reverse transcriptase (Invitrogen) and treated with DNAse I (Ambion) for 30 min. Then one-tenth of the produced cDNA was amplified by PCR using Taq DNA polymerase (Invitrogen) and two reporter sequence–specific primers. The PCR products were fluorescently labeled by primer extension with 6-arboxyfluorescein (6-FAM) 5'-labeled reporter sequence–specific primer (2 min at 95°C, 20 s at 68°C and 10 min at 72°C) and these products were digested with 5U of *Hpa*I (New England Biolabs) for 2h at 37°C. The fragments were purified using Qiaquick PCR columns (Qiagen), analyzed on an ABI 3130xL genetic analyzer (Applied Biosystems) with peak positions identified by using a set of X-rhodamine (ROX)-labeled MapMarker1000 molecular weight standards (BioVentures). The raw capillary electrophoresis data was processed using Attagraph software (Attagene).

Proprietary Elements:

Factorial™ is a novel pathway profiling technology trademarked and patented by Attagene, Inc.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote thyroid receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Attagene, Inc.
7020 Kit Creek Road Suite 260
Morrisville, NC 27560
Tel: (888)721-2121

Assay Publication Year:

2010

Assay Publication:

Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., & Houck, K. A. (2010). Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol, 23(3), 578-590. (PMID: 20143881)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Attagene TRANS2 Thyroid Receptor beta assay measures changes in thyroid-receptor β (TRβ) activation using the mammalian one-hybrid assay format, which monitored transcriptional

activation using TRβ ligand-binding domain (LBD) fused with the yeast GAL4 DNA-binding domain (DBD) transiently transfected into HepG2 cells with a 5X upstream activator sequence (UAS) reporter. Each individual trans-RTU system (receptor and reporter gene) was separately transfected into the cell, followed by pooling and plating of the transfected cells prior to detection. The presence of agonists/antagonists of TRβ alters the transactivation function of Gal4-NR and modulates reporter transcription. Following 24 hour incubation with compounds from the ToxCast chemical library, TF activity was reported via cDNA synthesis and RT-PCR of the fluorescently-labeled MRTU mRNA followed by quantitation by capillary electrophoresis. Half-maximal activity (IC_{50}) was determined for each chemical using changes in transcription factor activity expressed as log2 fold induction over DMSO controls in a single replicate, 8-point concentration response series. Cytotoxicity assessments were performed concurrently using a MTT tetrazolium assay to establish maximum tolerated concentrations (MTC) (see ATG_XTT_Cytotoxicity assay description for MTT procedures).

ATG_THRb_TRANS2 series assays consist of multiplexed (Factorial™) assay endpoints which assessed transcription factor activity using reporter transcription unit (RTU) quantitation in the HepG2 human liver hepatoma cell line. The multiple RTUs were constructed from essentially identical reporter gene sequences (representing a functionally inactivated fragment of human SEAP cDNA) and each tagged with unique *Hpa*I restriction cleavage sites at variable positions to allow discrimination of individual TF activities. When co-introduced into HepG2 cells, the RTUs produce reporter RNAs in amounts commensurate with the activities of the corresponding TFs present in a cell. This homogeneous construct creates a uniform environment for detecting the activity of many transcription factors simultaneously, and allowing for a more detailed assessment of xenobiotic impacts on the cellular regulatory network. ATG_THRb_TRANS2 is a MRTU Factorial™ assay reporting activity in exogenous, chimeric Gal4-TRβ proteins that bind to a GAL4-specific upstream activation sequence promoter (5X-UAS-TATA) and regulate the transcription of a TRβ-specific reporter sequence. Chemical-THRβ activity can be monitored by measuring changes in fluorescence relative to DMSO controls.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting thyroid hormone signaling pathways. The thyroid receptor mediates gene expression in response to T3 / T4, and modulates the activity for a wide variety of physiological processes. The ATG_THRb_TRANS2 assay used a hepatoma cell-based platform to monitor thyroid receptor alpha transcriptional activity and this assay is designed to help identify environmental compounds with a capacity for endocrine disrupting activity.

Method Development Reference:

Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., & Makarov, S. (2008). Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods, 5(3), 253-260. (PMID: 18297081)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.303
Neutral control median absolute deviation, by plate:	0.0385
Positive control well median response value, by plate:	NA
Positive control well median absolute deviation, by plate:	NA
Negative control well median, by plate:	NA

Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	NA
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	NA
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	NA
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	NA
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Transcription factor activity - nuclear steroid hormone receptor initiated pathway production of mRNA transcripts in response to ligand binding of thyroid receptor β.

Analytical Elements:

ATG_THRb_TRANS2_up readout data was analyzed in the positive (gain of signal) fitting direction using log2 fold-induction over DMSO controls which provide a baseline signal. Each chemical is run as a single replicate in a 6-point concentration series, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Thyroid receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRa1_TRANS_up
LTEA_HepaRG_THRSP_dn
LTEA_HepaRG_THRSP_up
NVS_NR_hTRa_Antagonist
Tox21_TR_LUC_GH3_Agonist
Tox21_TR_LUC_GH3_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.04 µM

Standard maximum concentration tested:	10.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.278
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.390

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Aden, D. P., et al. (1979). Nature 282: 615-616. (PMID: 233137)

[2] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1581-1591. (PMID: 17637504)

[3] Hewitt, N. and P. Hewitt (2004). Xenobiotica 34(3): 243-256. (PMID: 15204697)

[4] Guo, L., et al. (2010). Drug Metab Disposition 39(3): 528-538. (PMID: 21149542)

[5] Westerink, W. M. and W. G. Schoonen (2007). Toxicol In Vitro 21(8): 1592-1602. (PMID: 17716855)

[6] Walle, T., et al. (2000). Drug Metab Disposition 28(9): 1077-1082. (PMID: 10950852)

[7] Hart, S. N., et al. (2010). Drug Metab Disposition 38(6): 988-994. (PMID: 20228232)

[8] Boehme, K., et al. (2010). Toxicol Lett 198(2): 272-281. (PMID: 20655369)

[9] Adachi, T., et al. (2007). J Exper Therap Oncol 6(4): 335-348. (PMID: 18038766)

[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
GST, Glutathione S-Transferase
MRTU, Multiple Reporter Transcription Unit

NR, Nuclear Receptors
RTU, Reporter Transcription Unit
SEAP, Secreted Embryonic Alkaline Phosphatase
SULT, Sulfotransferases
T3, Triiodothyronine
T4, Thyroxine
TF, Transcription Factor
TH, Thyroid Hormone
TR, Thyroid Receptor
UGT, UDP-Glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

20 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 890

CEETOX_H295R_11DCORT_dn

Assay Name: CeeTox H295R High-throughput Steroidogenesis Assay – 11-Deoxycortisol Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

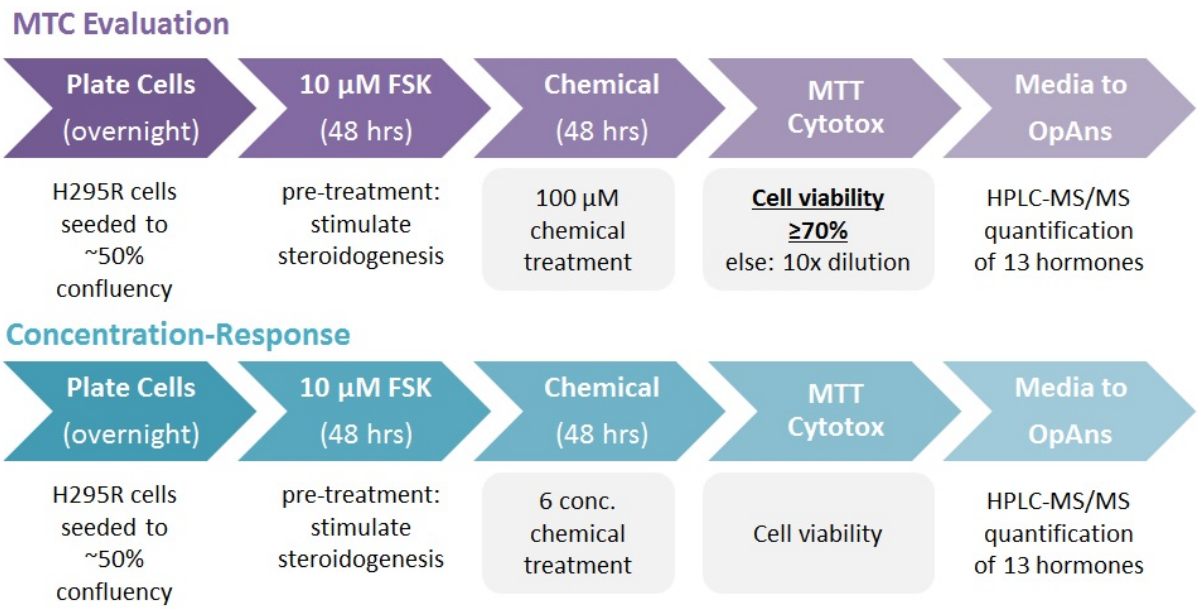

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R 11-deoxycortisol assay was used to screen a large chemical library for changes in 11-deoxycortisol levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for 11-deoxycortisol quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of 11-deoxycortisol from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 11-deoxycortisol were reported as 5 ng/mL and 1000 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 5.0% and accuracy of 101.7% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

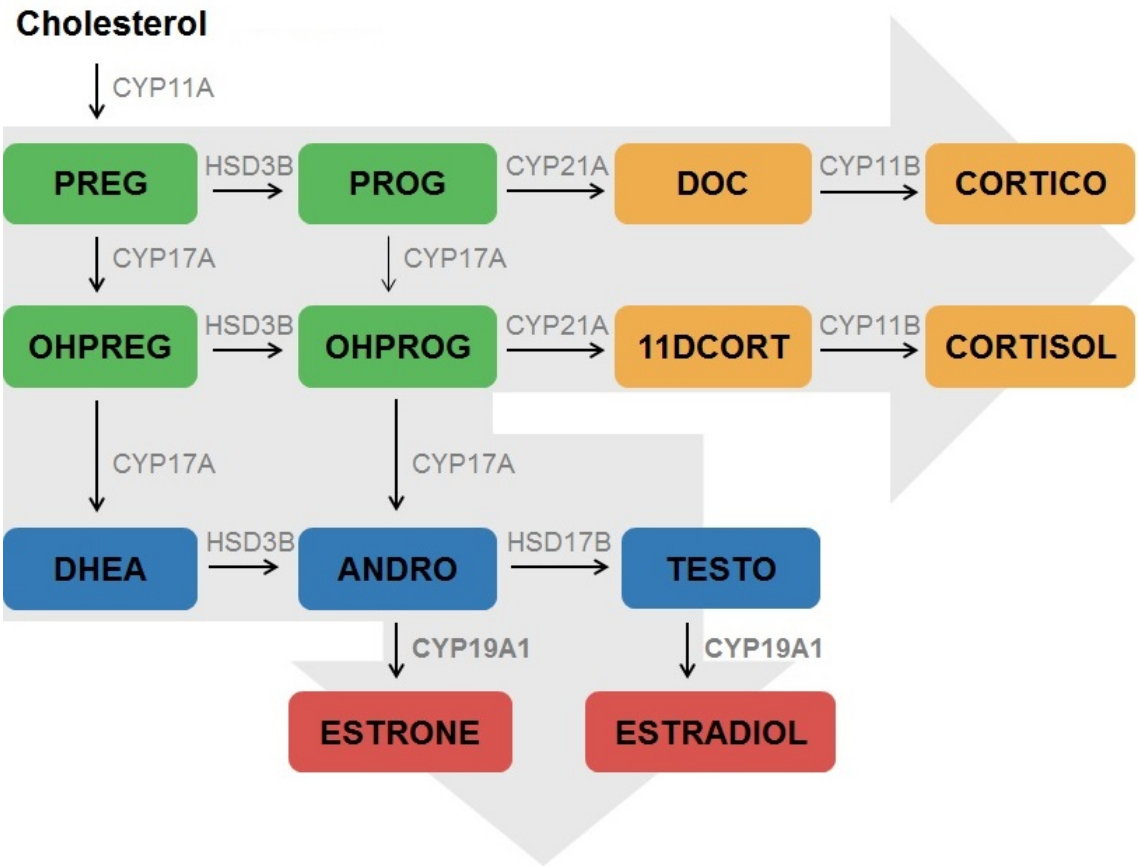

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 15 for 11DCORT. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -27 for 11DCORT. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of 11-deoxycortisol following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_11DCORT_dn readout data was analyzed in the negative (loss of signal) fitting direction, using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in 11-deoxycortisol activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. 11-deoxycortisol inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change, which was approximately a 1.50 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_11-DEOXYCORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn

CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For 11DCORT, the $r^2 = 0.82$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)

[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information (existing annotations):

Assay Endpoint ID: 891

CEETOX_H295R_11DCORT_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – 11-deoxycortisol Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

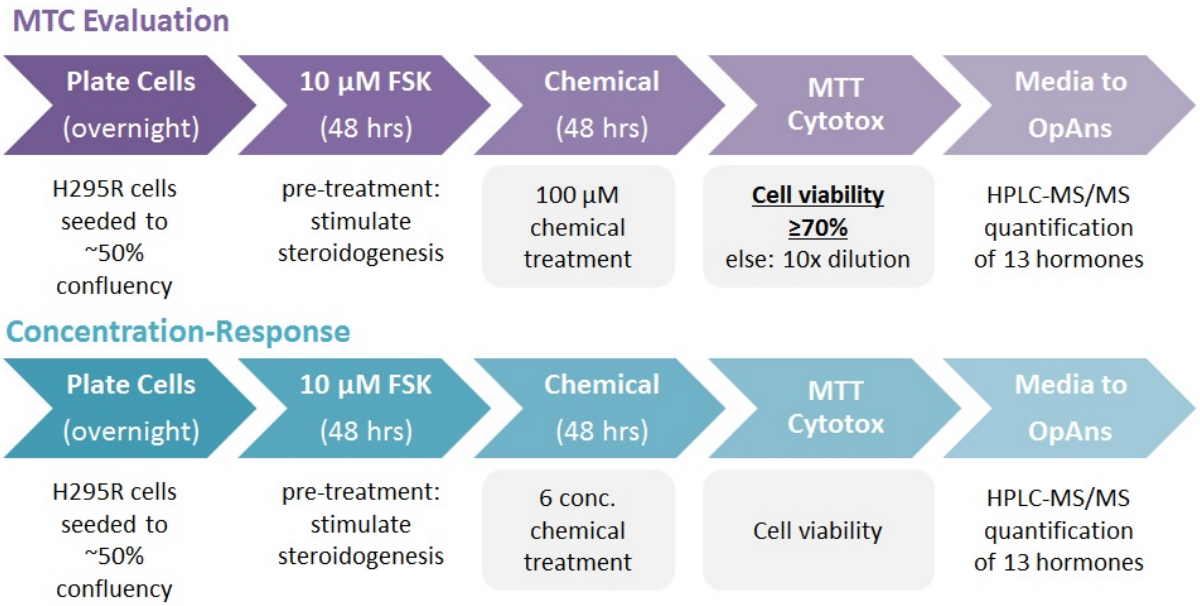

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R 11-deoxycortisol assay was used to screen a large chemical library for changes in 11-deoxycortisol levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application a three-tiered screening approach was employed The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for 11-deoxycortisol quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of 11-deoxycortisol from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 11-deoxycortisol were reported as 5 ng/mL and 1000 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 5.0% and accuracy of 101.7% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis) resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

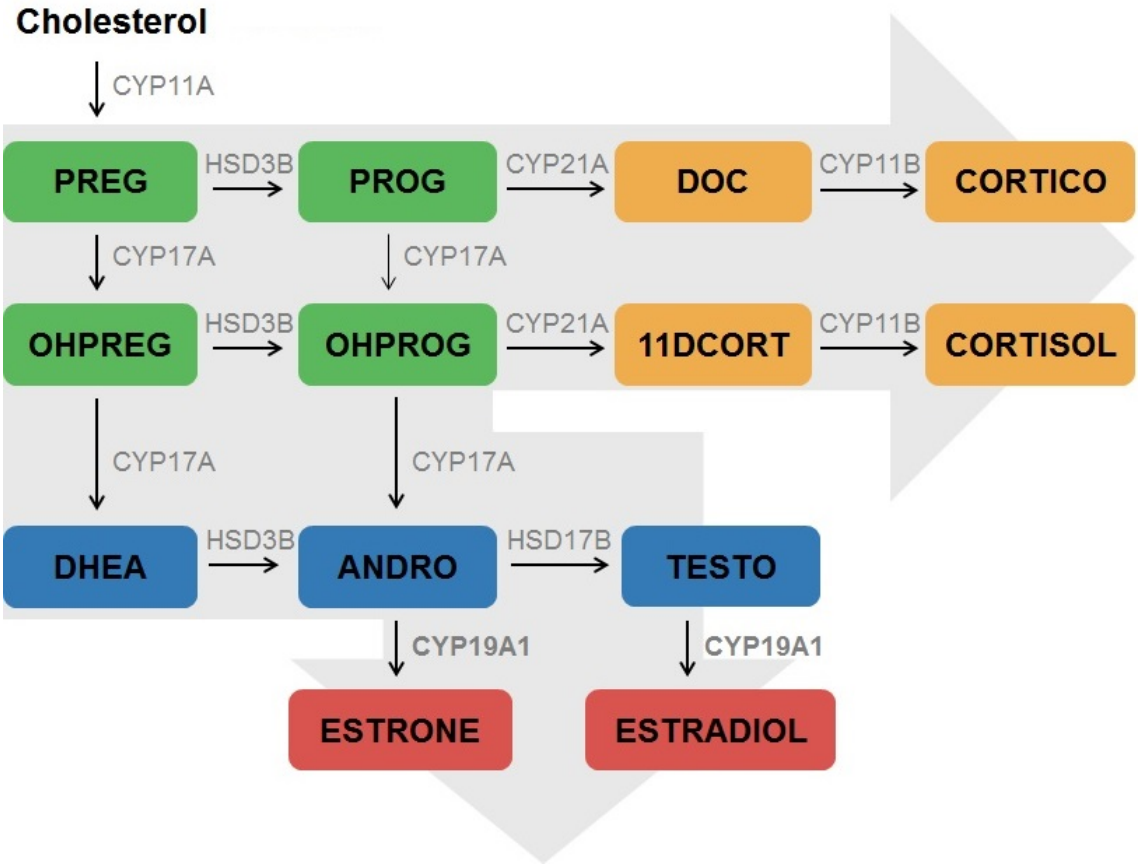

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 15 for 11DCORT. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -27 for 11DCORT. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of 11-deoxycortisol following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_11DCORT_up readout data was analyzed in the positive (gain of signal) fitting direction, using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in 11-deoxycortisol activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$ transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. 11-deoxycortisol stimulation was determined based on a chemical fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change which was approximately 1.50 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_11-DEOXYCORTISOL_dn
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up

CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For 11DCORT, the $r^2 = 0.82$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)

[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

Assay Endpoint ID: 896

CEETOX_H295R_ANDR_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Androstenedione Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

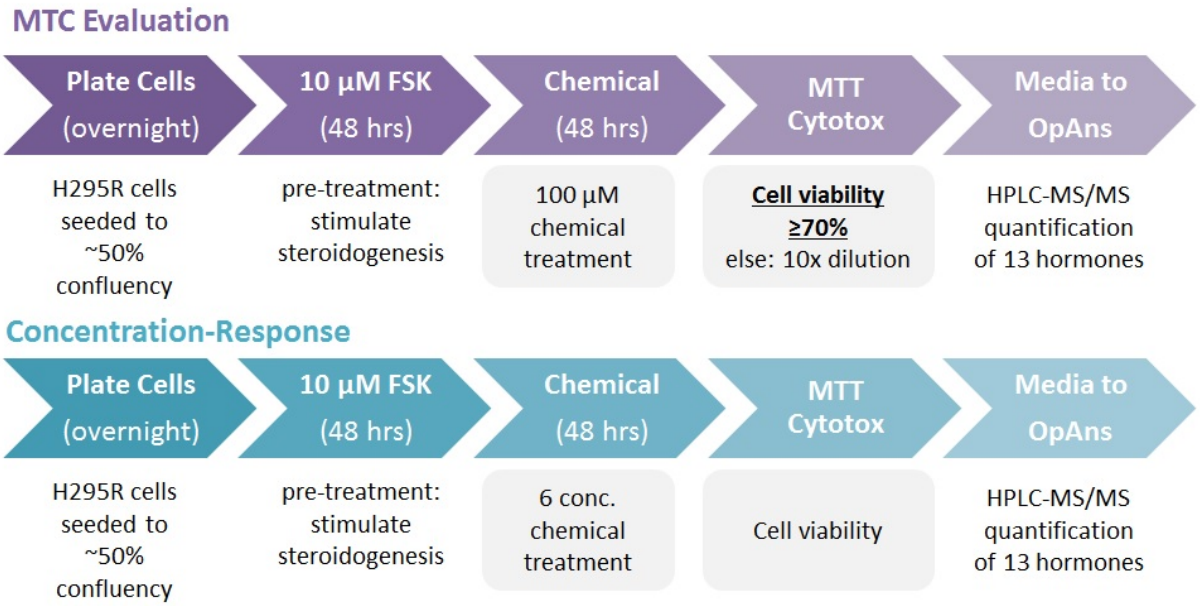

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

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Assay Publication Year:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R androstenedione assay was used to screen a large chemical library for changes in androstenedione levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for androstenedione quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of androstenedione from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for androstenedione were reported as 1 ng/mL and 200 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 4.7% and accuracy of 99.9% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

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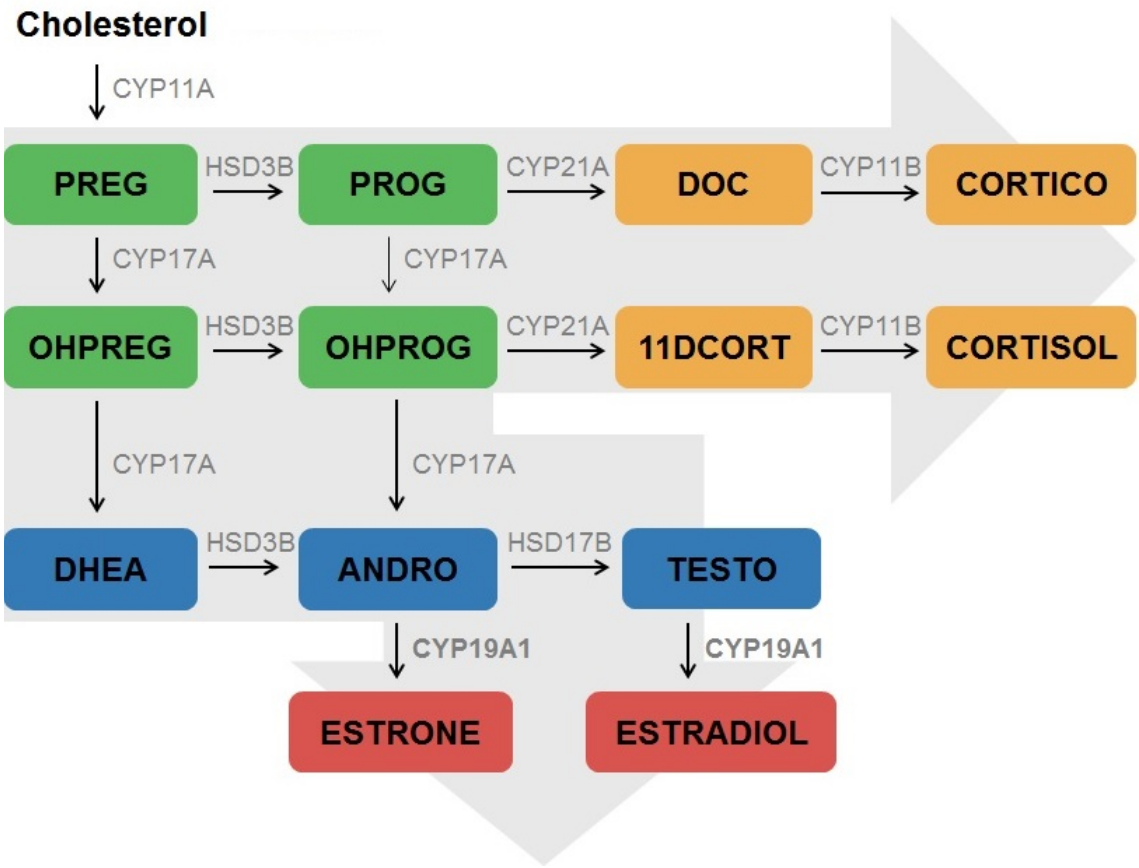

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

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Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.68 for androstenedione. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.84 for androstenedione. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 11 for androstenedione. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -19 for androstenedione. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of androstenedione following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_ANDROSTENEDIONE_dn readout data was analyzed in the negative (loss of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in androstenedione activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Androstenedione inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change; which was approximately a 1.70 fold-change cut-off); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up

CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For androstenedione, the $r^2 = 0.81$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

13 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

Assay Endpoint ID: 897

CEETOX_H295R_ANDR_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Androstenedione Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

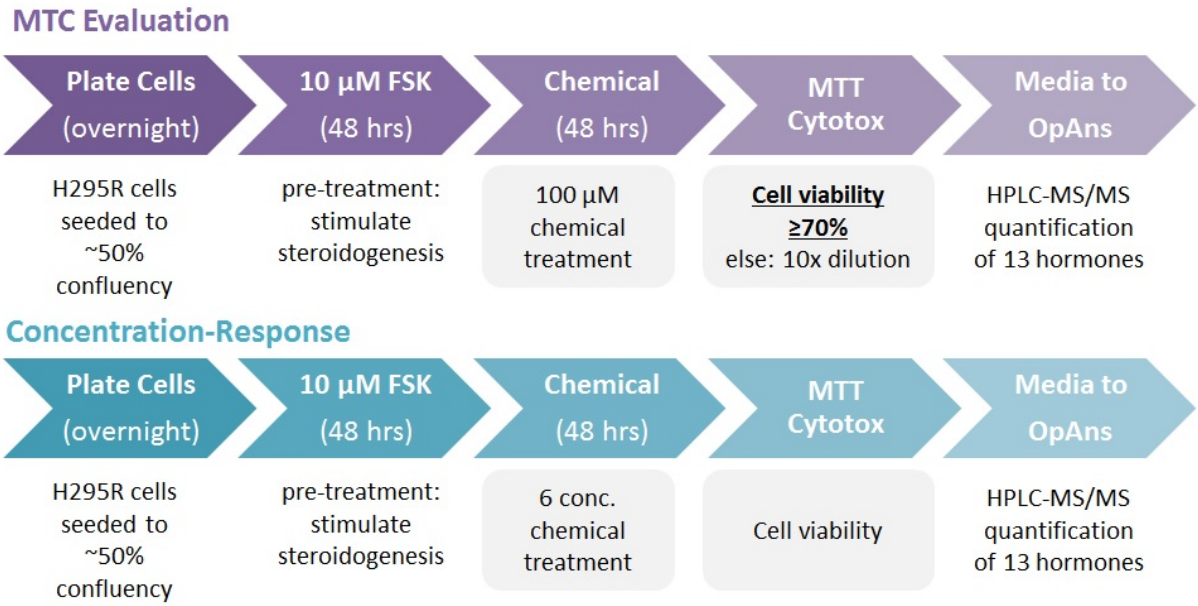

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R androstenedione assay was used to screen a large chemical library for changes in androstenedione levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for androstenedione quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of androstenedione from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for androstenedione were reported as 1 ng/mL and 200 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 4.7% and accuracy of 99.9% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

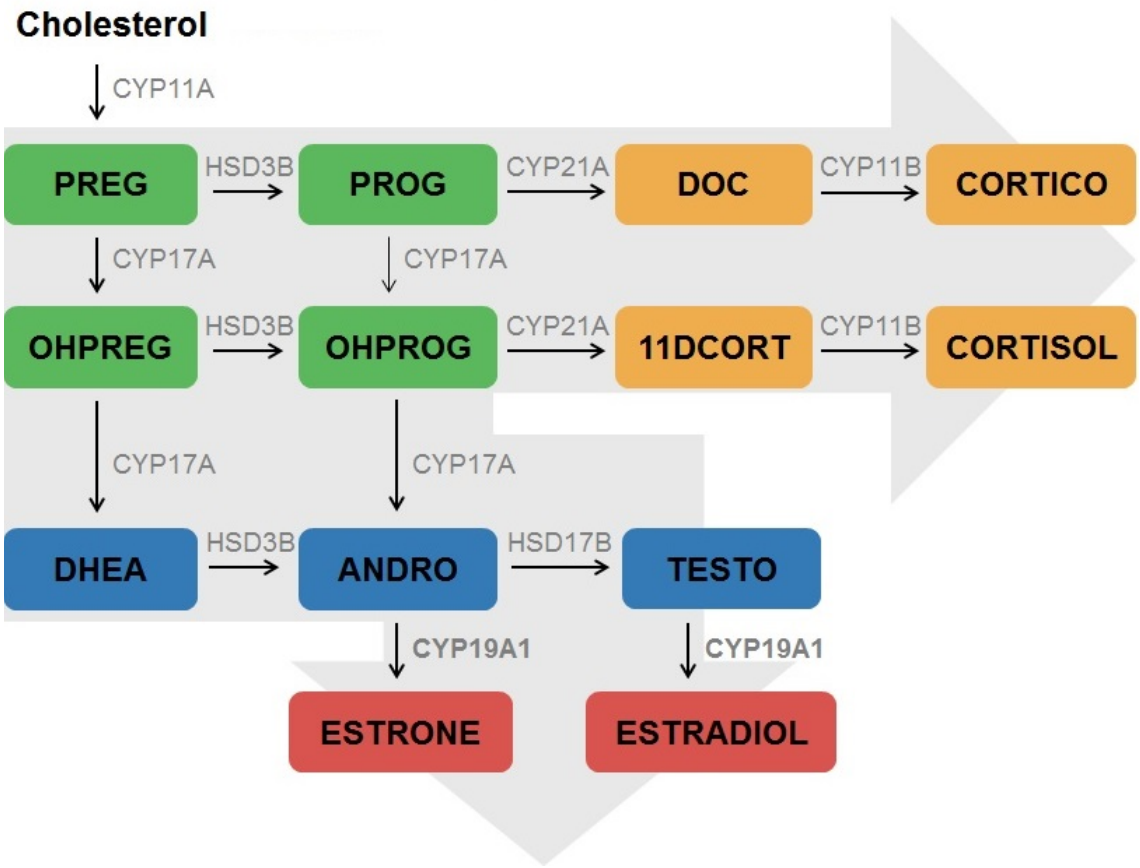

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.68 for androstenedione. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.84 for androstenedione. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

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3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of androstenedione following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_ANDROSTENEDIONE_up readout data was analyzed in the positive (gain of signal) fitting direction using the DMSO controls (baseline signal), and was reported as log2 fold-change increase in androstenedione activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androstenedione stimulation was determined based on a chemical fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change; in the androstenedione assay this created a 1.70 fold-change cut-off); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
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3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For androstenedione, the $r^2 = 0.81$. These values demonstrate that the assay is highly reproducible.

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4. Assay Documentation

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[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
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4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
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MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

Assay Endpoint ID: 898

CEETOX_H295R_CORTIC_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Corticosterone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

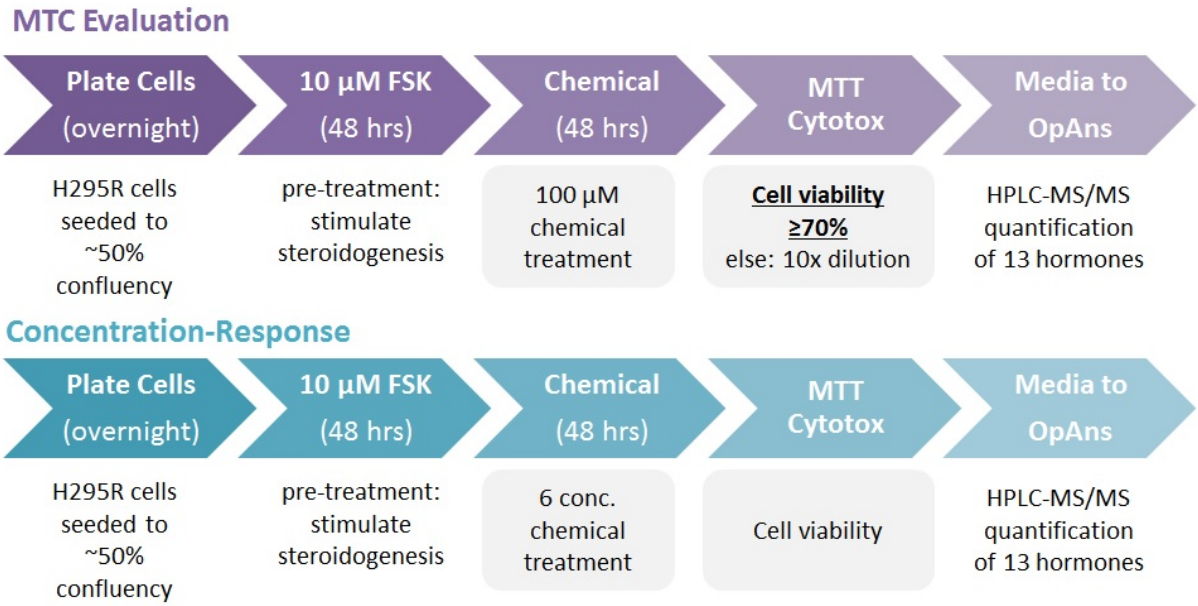

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Corticosterone assay was used to screen a large chemical library for changes in Corticosterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for corticosterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of corticosterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10 µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for corticosterone were reported as 0.5 ng/mL and 100 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 4.7% and accuracy of 100.5% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

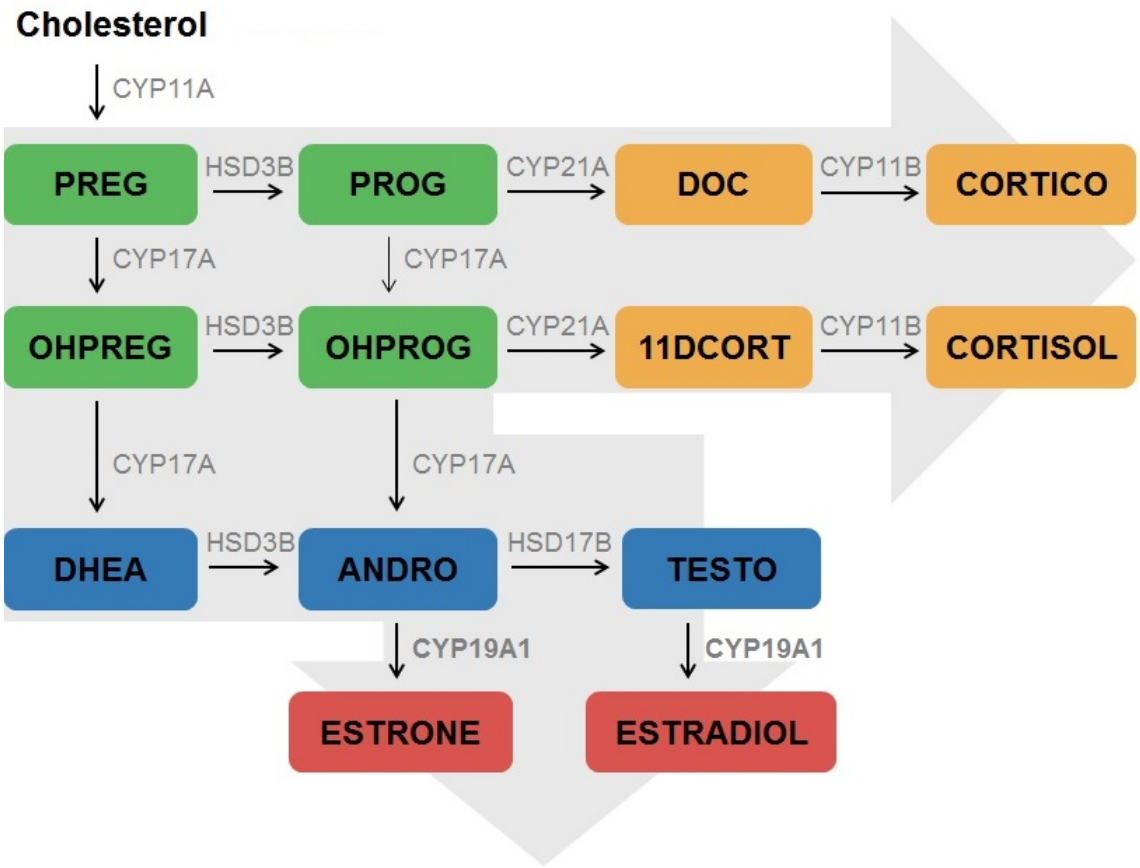

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18. SSMD-prochloraz ranged from -27-7. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of corticosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_CORTIC_dn readout data was analyzed in the negative (loss of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in corticosterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Corticosterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn

CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.

[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

Assay Endpoint ID: 899

CEETOX_H295R_CORTIC_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Corticosterone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified/adapted version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, seeking ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC. If a test chemical altered the levels of at least 4 different hormones, the chemical was selected for follow-up concentration-response evaluation to establish whether changes in hormone levels were concentration-dependent. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones such as androgens and estrogens.

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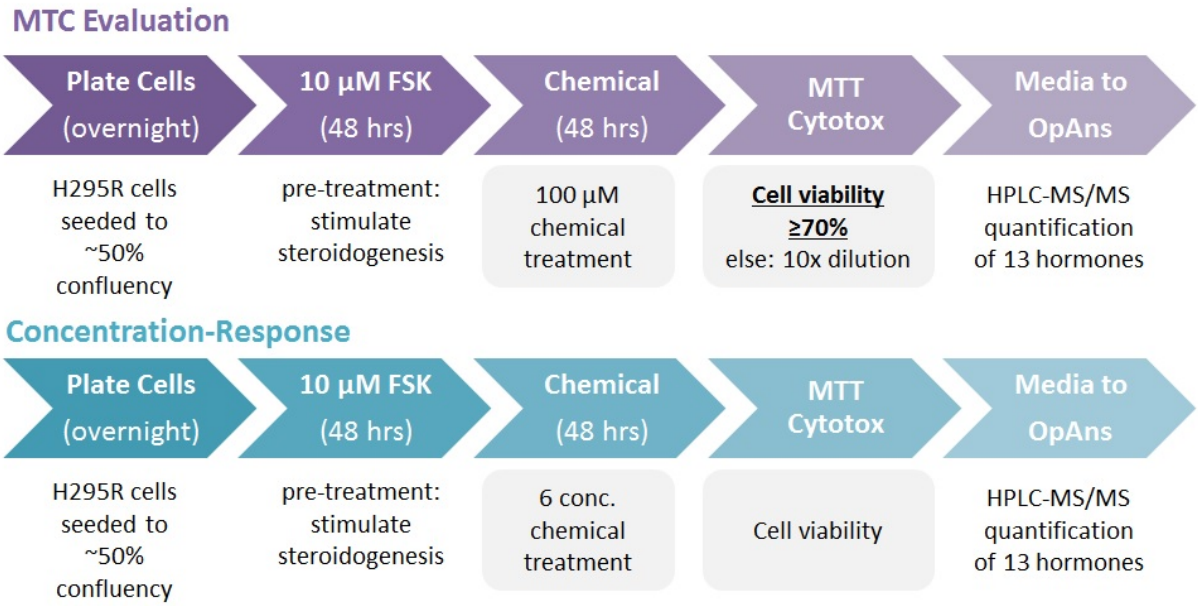
H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.



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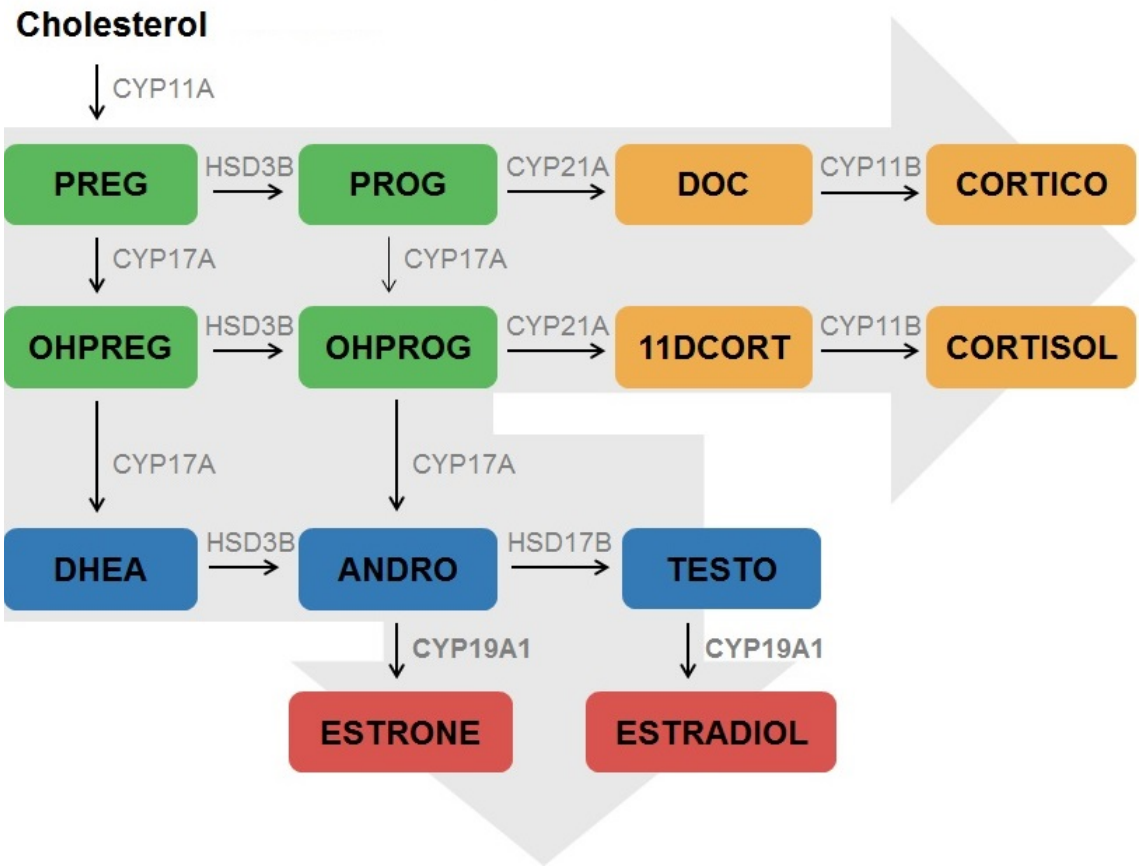

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

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Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18. SSMD-prochloraz ranged from -27-7. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of corticosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_CORTIC_up readout data was analyzed in the positive (gain of signal) fitting direction using the DMSO controls as a baseline signal, and was reported as log2 fold-change increase in corticosterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Corticosterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn

CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)

[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

Assay Endpoint ID: 900

CEETOX_H295R_CORTISOL_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Cortisol Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

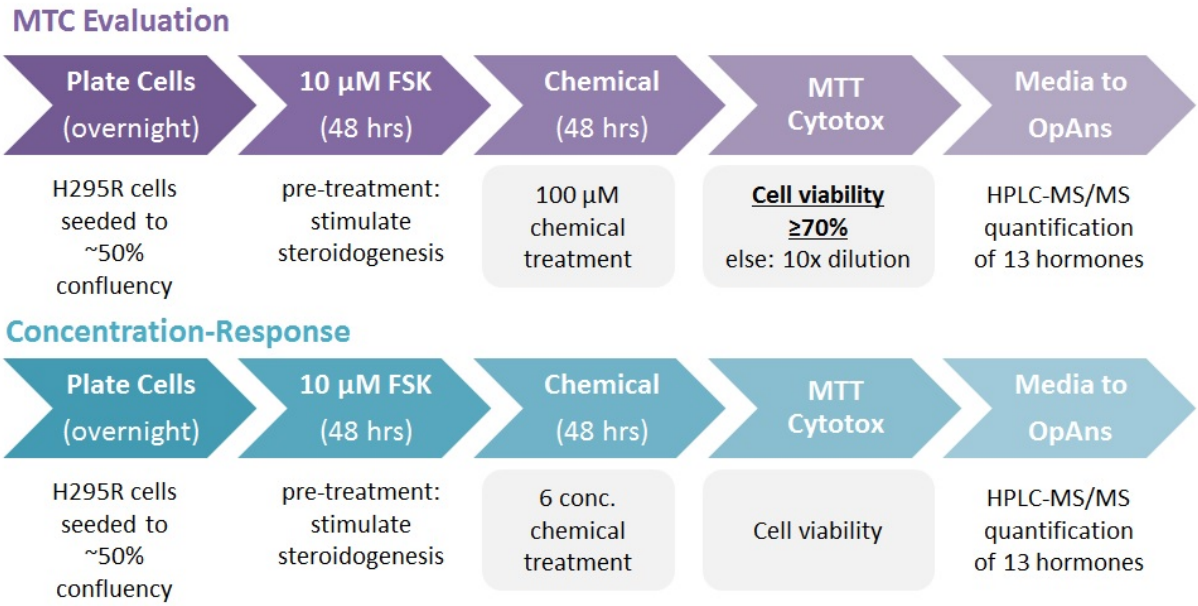

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Cortisol assay was used to screen a large chemical library for changes in cortisol levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for cortisol quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of cortisol from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for cortisol were reported as 0.5 ng/mL and 100 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 3.3% and accuracy of 99.7% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

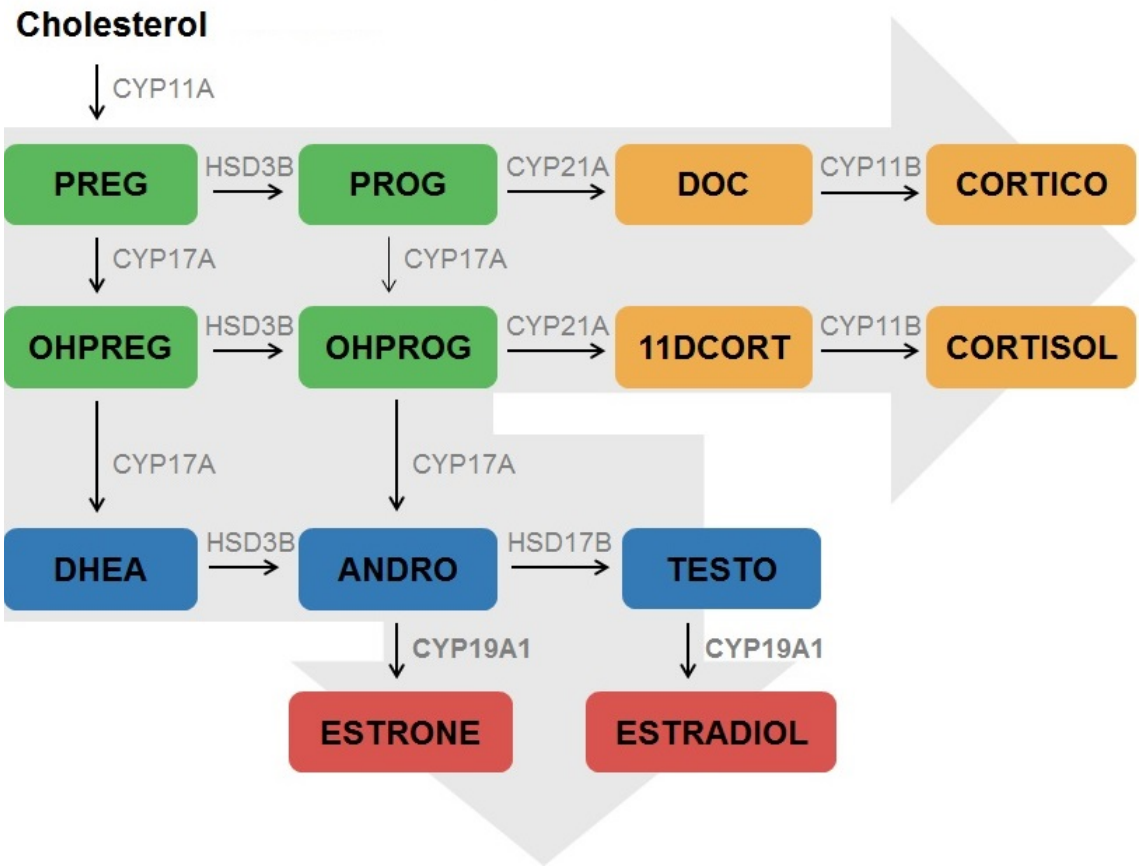

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.67 for cortisol. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.83 for cortisol. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 11 for cortisol. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -18 for cortisol. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of cortisol following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_CORTISOL_dn readout data was analyzed in the negative (loss of signal) fitting direction using the DMSO controls as a baseline signal, and was reported as log2 fold-change increase in cortisol activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Cortisol inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change, which was approximately a 1.98 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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CEETOX_H295R_TESTO_up

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Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For cortisol, the $r^2 = 0.78$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

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[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
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R^2, correlation coefficient, R-squared
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Z', Robust Z-prime

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

Assay Endpoint ID: 901

CEETOX_H295R_CORTISOL_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Cortisol Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

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Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

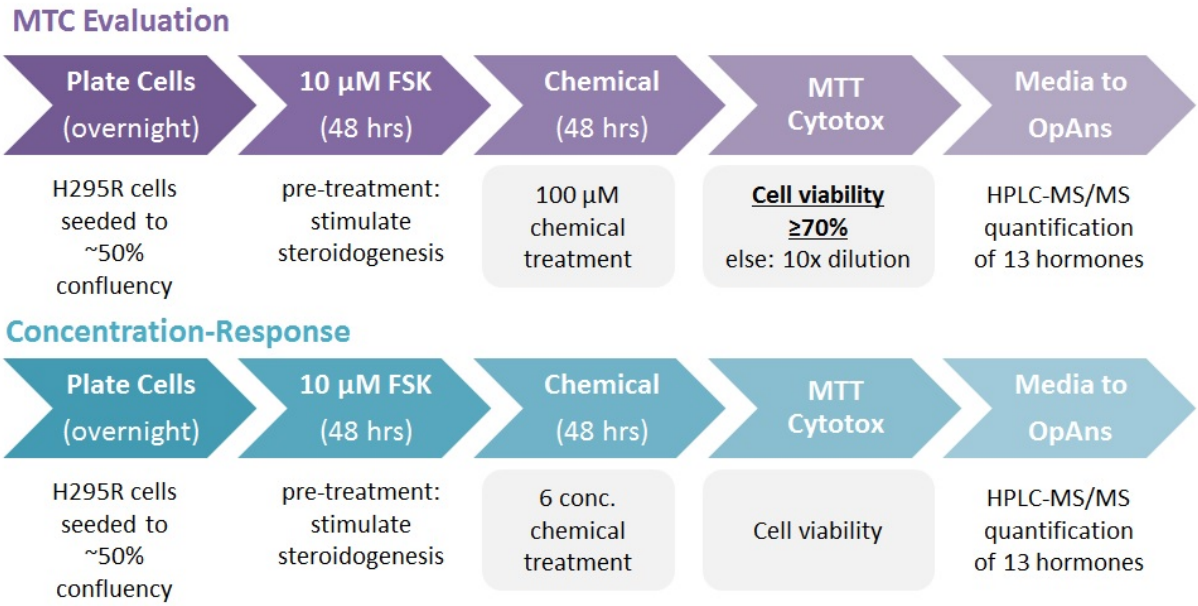

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Cortisol assay was used to screen a large chemical library for changes in cortisol levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for cortisol quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of cortisol from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for cortisol were reported as 0.5 ng/mL and 100 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 3.3% and accuracy of 99.7% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

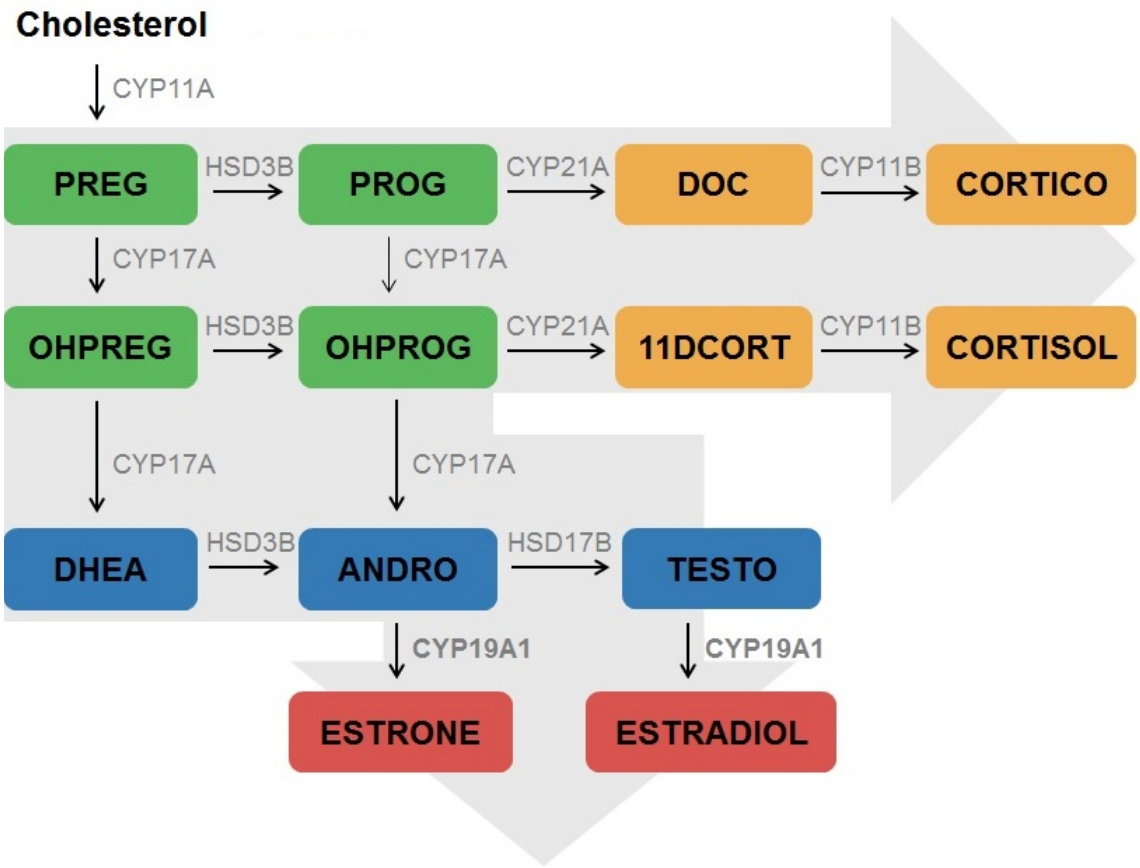

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.67 for cortisol. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.83 for cortisol. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 11 for cortisol. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -18 for cortisol. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of cortisol following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_CORTISOL_up readout data was analyzed in the positive (gain of signal) fitting direction using the DMSO controls as a baseline signal, and was reported as log2 fold-change increase in cortisol activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Cortisol inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change, which was approximately a 1.98 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_DHEA_dn

CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For cortisol, the $r^2 = 0.78$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

CEETOX_H295R_DHEA_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – DHEA Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

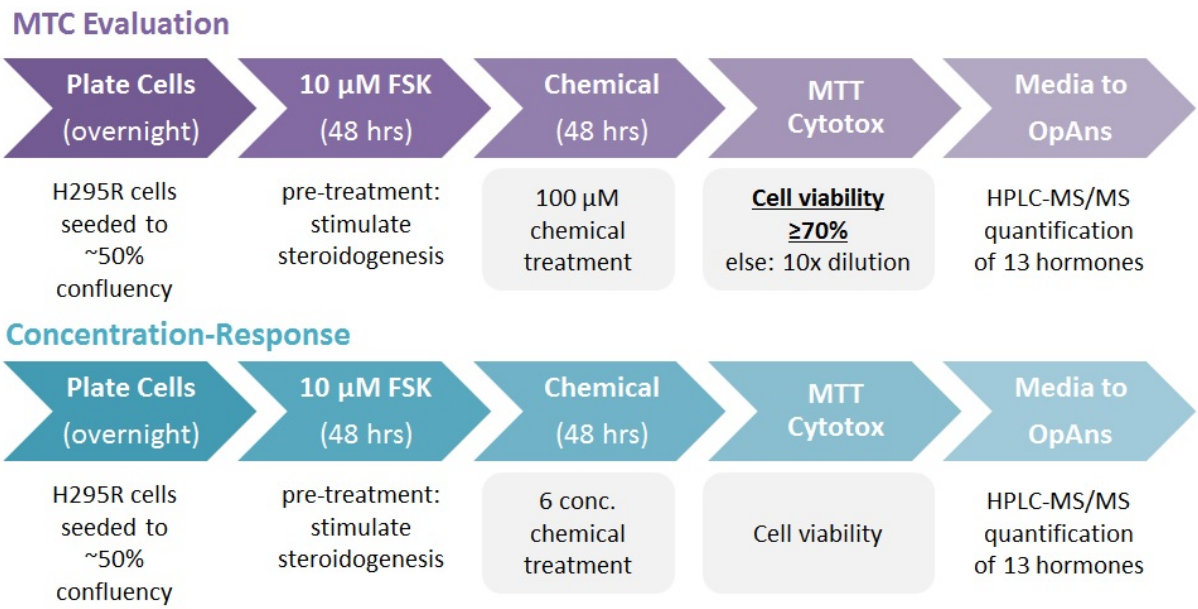

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

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Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
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Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Dehydroepiandrosterone (DHEA) assay was used to screen a large chemical library for changes in DHEA levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for DHEA quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of DHEA from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10 µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for DHEA were reported as 3 ng/mL and 600 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 4.0% and accuracy of 100.1% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

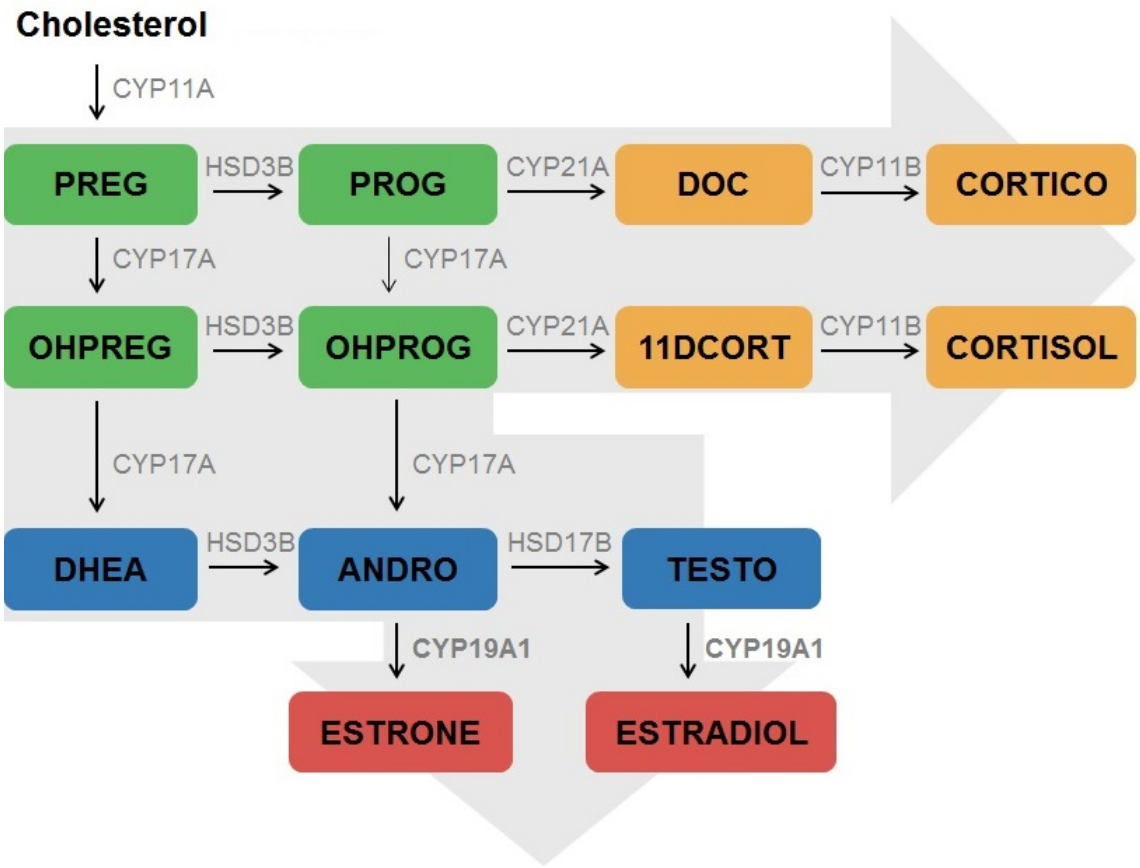

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

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USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18. SSMD-prochloraz ranged from -27-7. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of DHEA following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_DHEA_dn readout data was analyzed in the negative (loss of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in DHEA activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. DHEA inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn

CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DHEA, Dehydroepiandrosterone
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

13 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

CEETOX_H295R_DHEA_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – DHEA Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

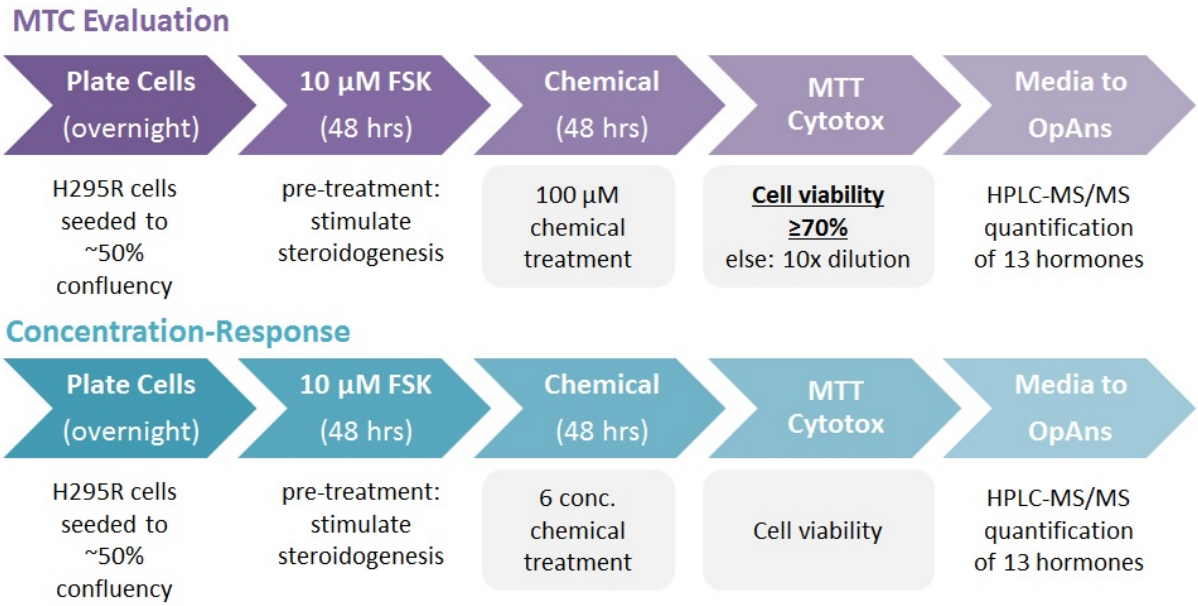

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18. SSMD-prochloraz ranged from -27-7. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Dehydroepiandrosterone (DHEA) assay was used to screen a large chemical library for changes in DHEA levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for DHEA quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of dehydroepiandrosterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for DHEA were reported as 3 ng/mL and 600 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 4.0% and accuracy of 100.1% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by

CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

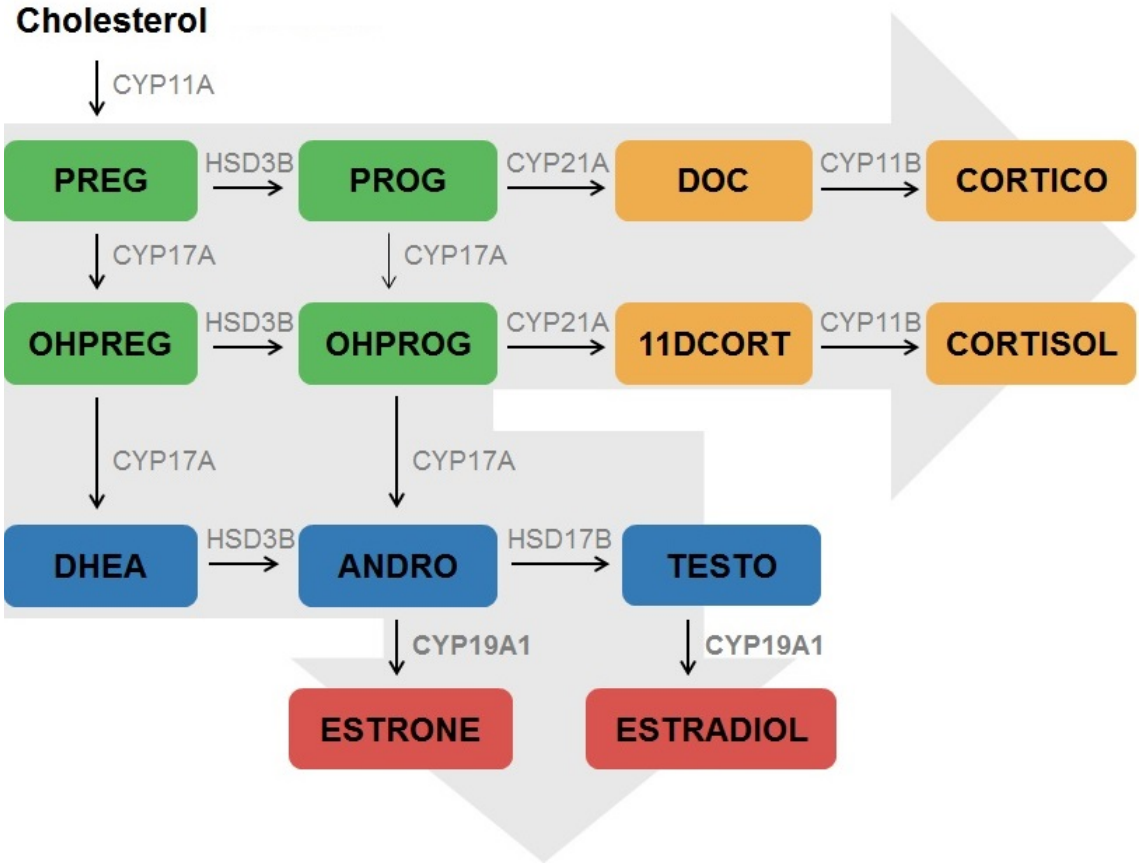

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of dehydroepiandrosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_DHEA_up readout data was analyzed in the positive (gain of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in DHEA activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. DHEA stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
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CEETOX_H295R_OHPROG_dn

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CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
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3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. These values demonstrate that the assay is highly reproducible.

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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
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[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
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4.2. Abbreviations and Definitions

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MTC, Maximum Tolerated Concentration
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R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
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4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Supporting Information:

CEETOX_H295R_DOC_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Deoxycorticosterone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

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Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

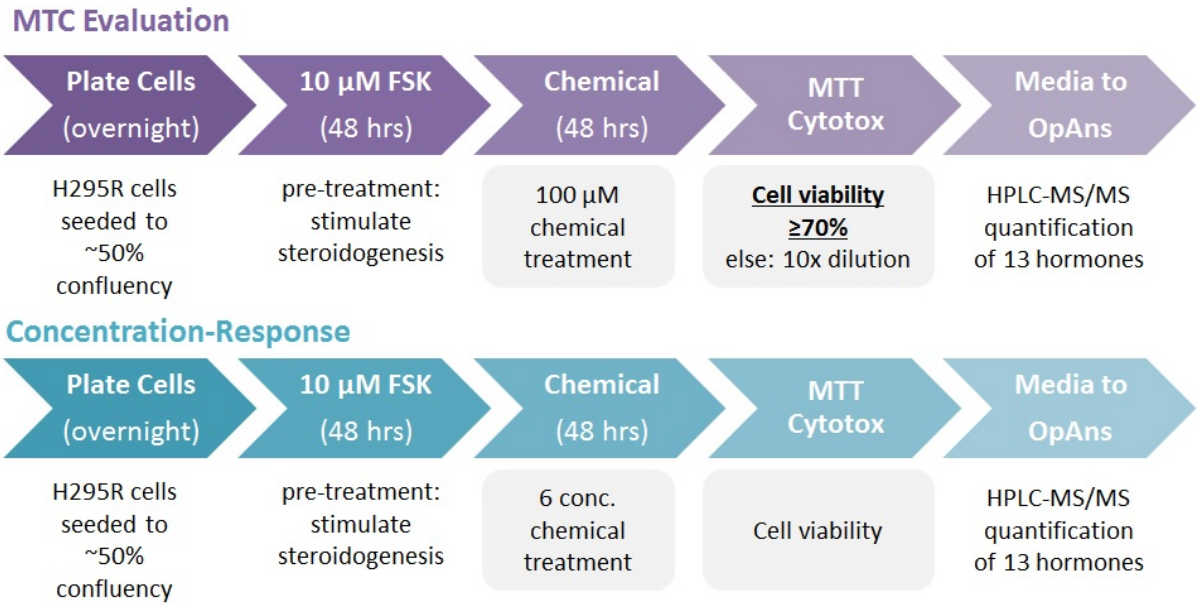

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

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Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

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Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

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The CeeTox H295R 11-deoxycorticosterone assay was used to screen a large chemical library for changes in deoxycorticosterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for deoxycorticosterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of deoxycorticosterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 11-deoxycorticosterone were reported as 0.5 ng/mL and 100 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 3.7% and accuracy of 99.9% (Karmaus *et al.*, 2016, Table 1).

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To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

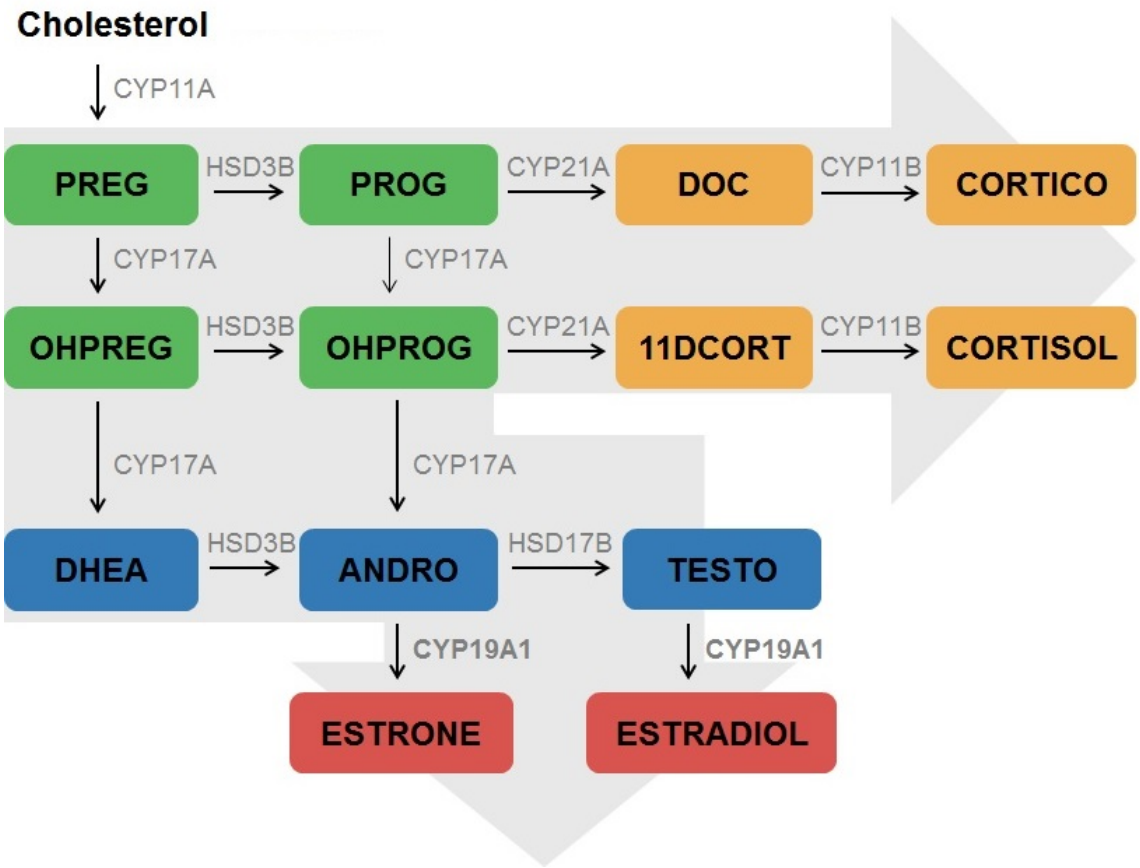

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.65 for deoxycorticosterone. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.51 for deoxycorticosterone. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 10 for deoxycorticosterone. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of 7 for deoxycorticosterone. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of 11-deoxycorticosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_DOC_dn readout data was analyzed in the negative (loss of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in deoxycorticosterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Deoxycorticosterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change, which was approximately a 1.90 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_up

CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
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CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For deoxycorticosterone, the $r^2 = 0.45$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_DOC_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Deoxycorticosterone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

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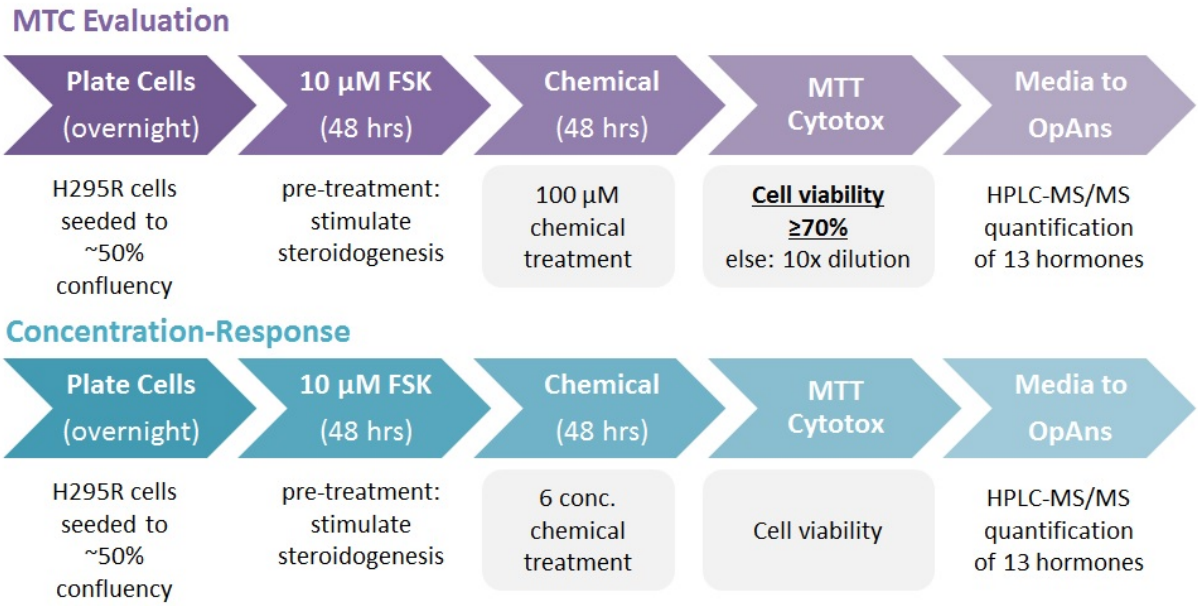

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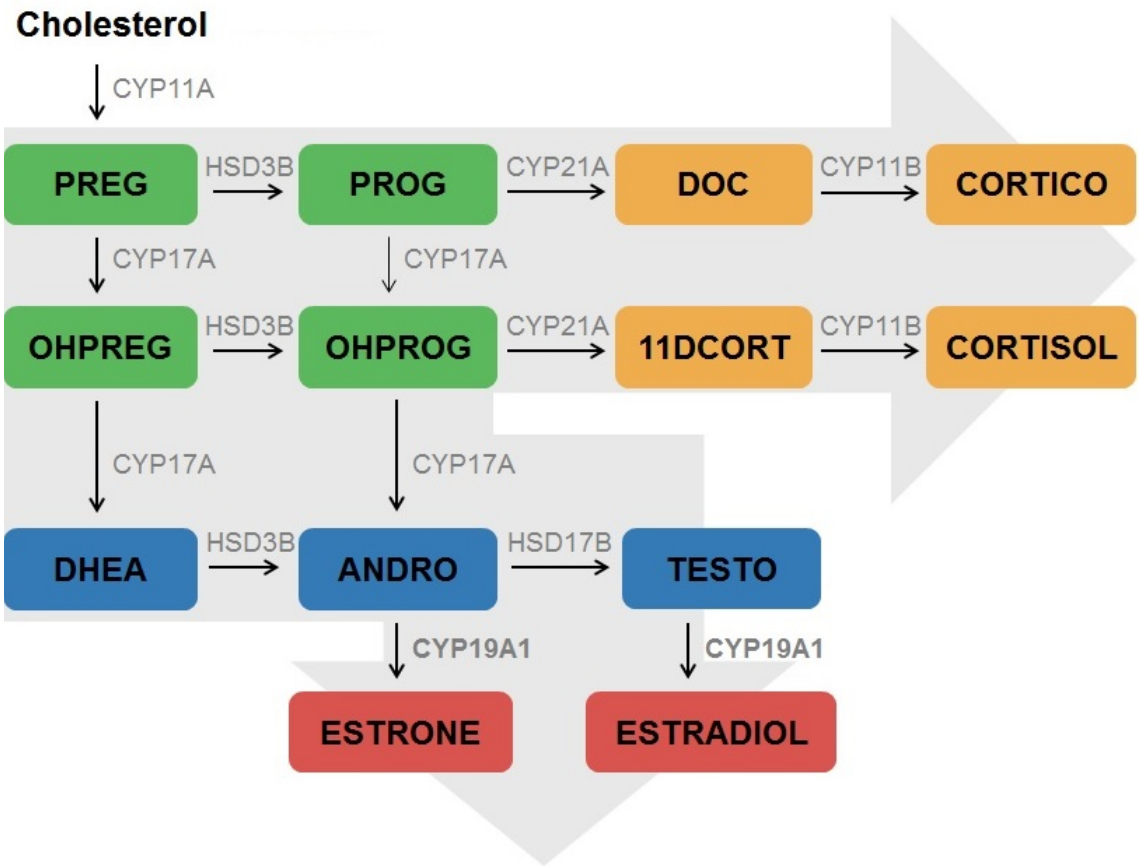

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.65 for deoxycorticosterone. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.51 for deoxycorticosterone. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 10 for deoxycorticosterone. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of 7 for deoxycorticosterone. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of 11-deoxycorticosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_DOC_up readout data was analyzed in the positive (gain of signal) fitting direction using the DMSO controls as a baseline signal, and was reported as log2 fold-change increase in deoxycorticosterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Deoxycorticosterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change, which was approximately a 1.90 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up

CEETOX_H295R_DOC_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For deoxycorticosterone, the $r^2 = 0.45$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 906

CEETOX_H295R_ESTRADIOL_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Estradiol Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

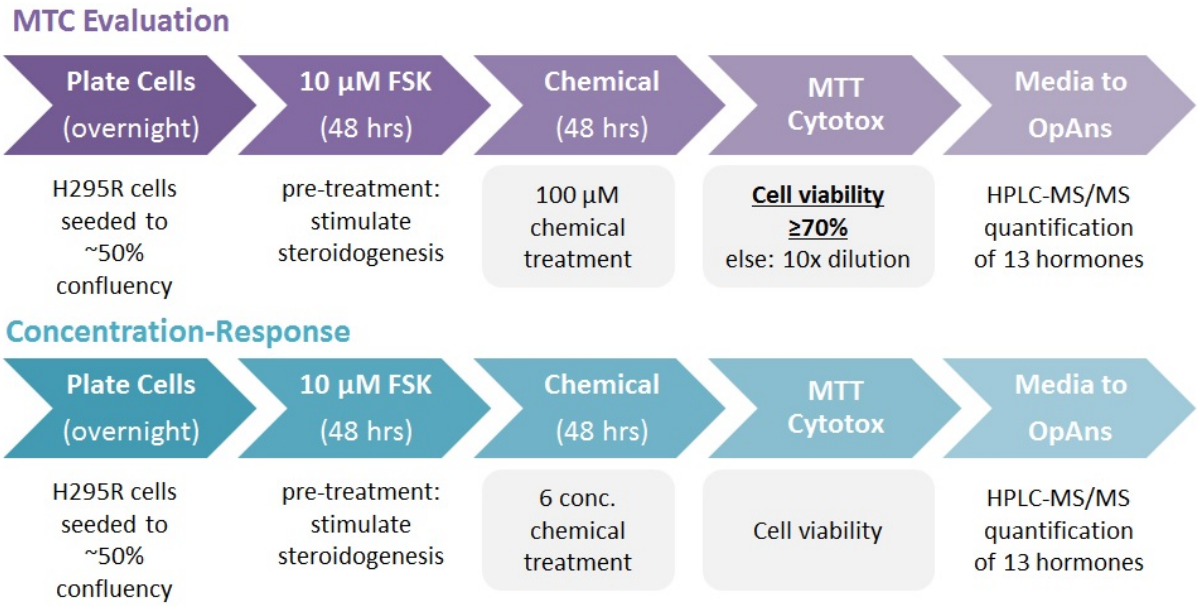

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R estradiol assay was used to screen a large chemical library for changes in estradiol levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for estradiol quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of estradiol from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for estradiol were reported as 0.03 ng/mL and 6 ng/mL, respectively, using 0.4, 1, and 16 ng/mL standards, with precision of 6.3% and accuracy of 101.4% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

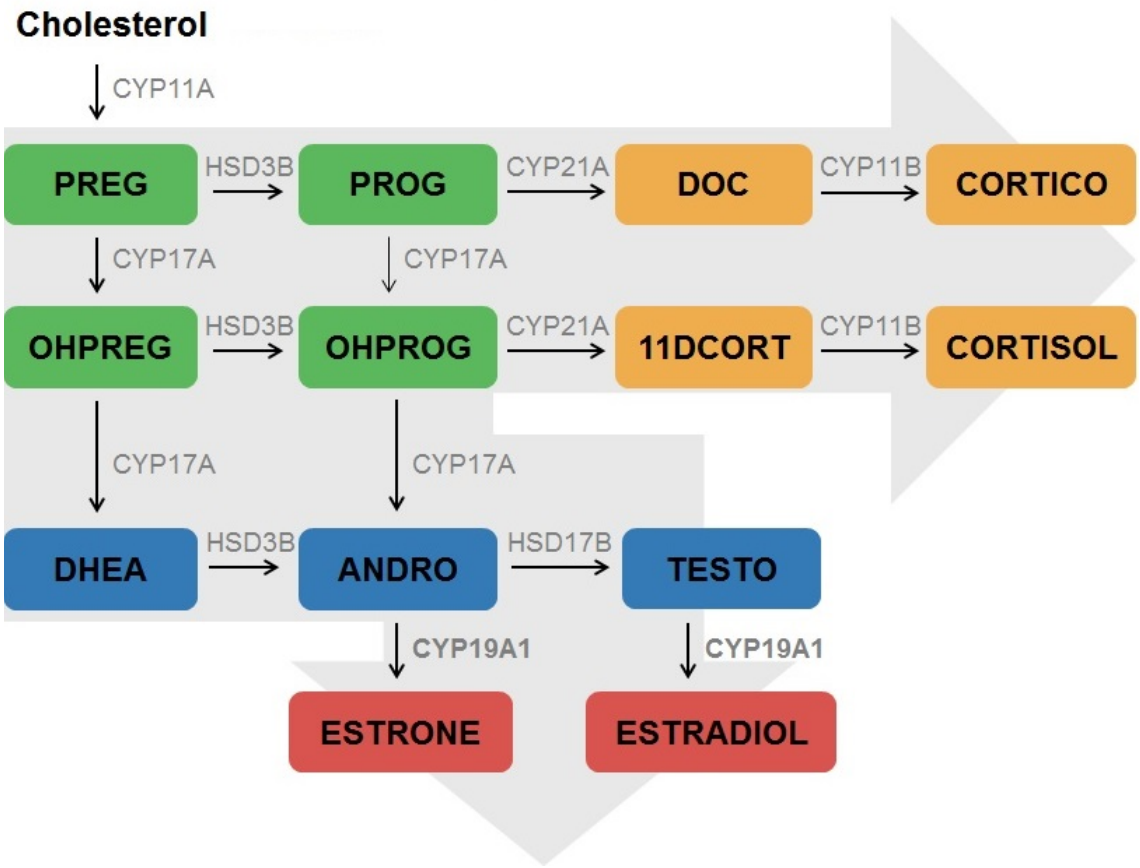

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

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Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.73 for estradiol. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.72 for estradiol. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 13 for estradiol. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -11 for estradiol. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of estradiol following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_ESTRADIOL_dn readout data was analyzed in the negative (loss of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in estradiol activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Estradiol inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change, which was approximately a 2.05 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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4. Assay Documentation

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[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
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[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
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4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
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DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
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MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

13 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 907

CEETOX_H295R_ESTRADIOL_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Estradiol Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

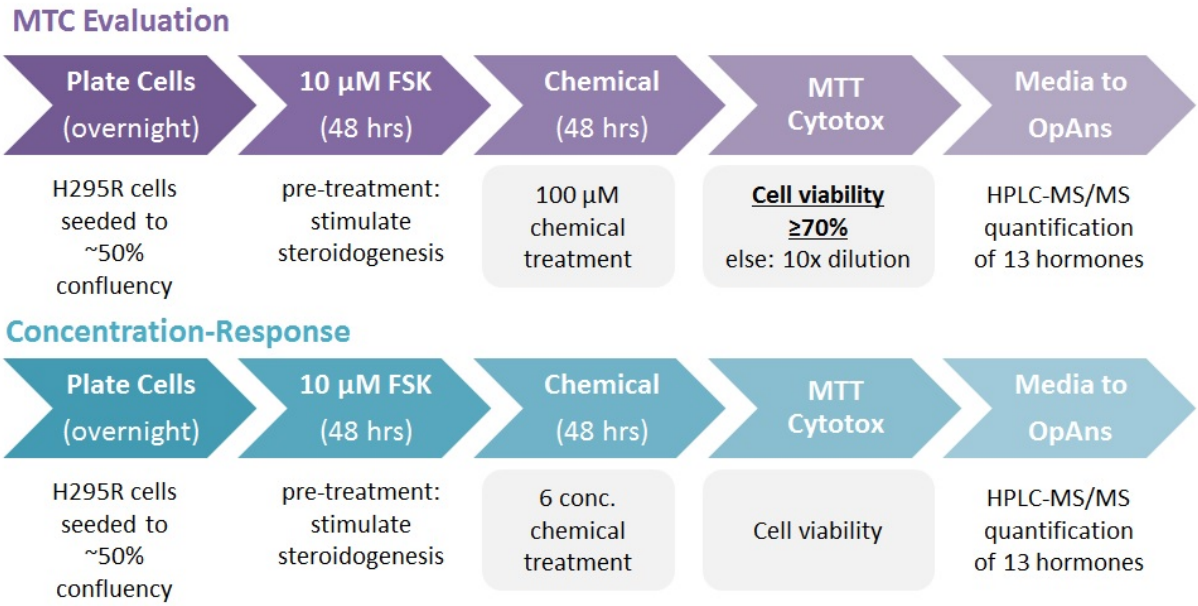

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R estradiol assay was used to screen a large chemical library for changes in estradiol levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for estradiol quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of estradiol from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for estradiol were reported as 0.03 ng/mL and 6 ng/mL, respectively, using 0.4, 1, and 16 ng/mL standards, with precision of 6.3% and accuracy of 101.4% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

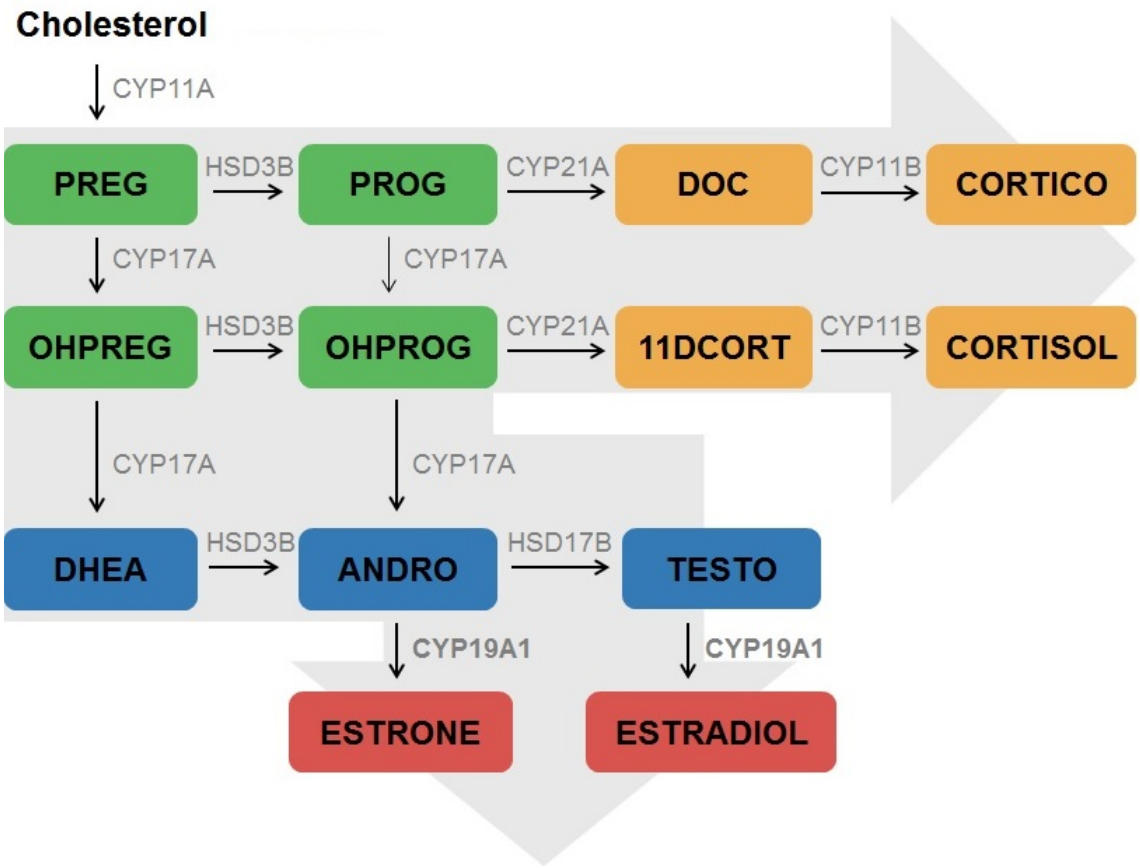

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.73 for estradiol. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.72 for estradiol. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 13 for estradiol. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -11 for estradiol. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of estradiol following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_ESTRADIOL_up readout data was analyzed in the positive (gain of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in estradiol activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Estradiol stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change, which was approximately a 2.05 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up

CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For estradiol, the $r^2 = 0.64$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_ESTRONE_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Estrone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

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Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

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Basic Procedure:

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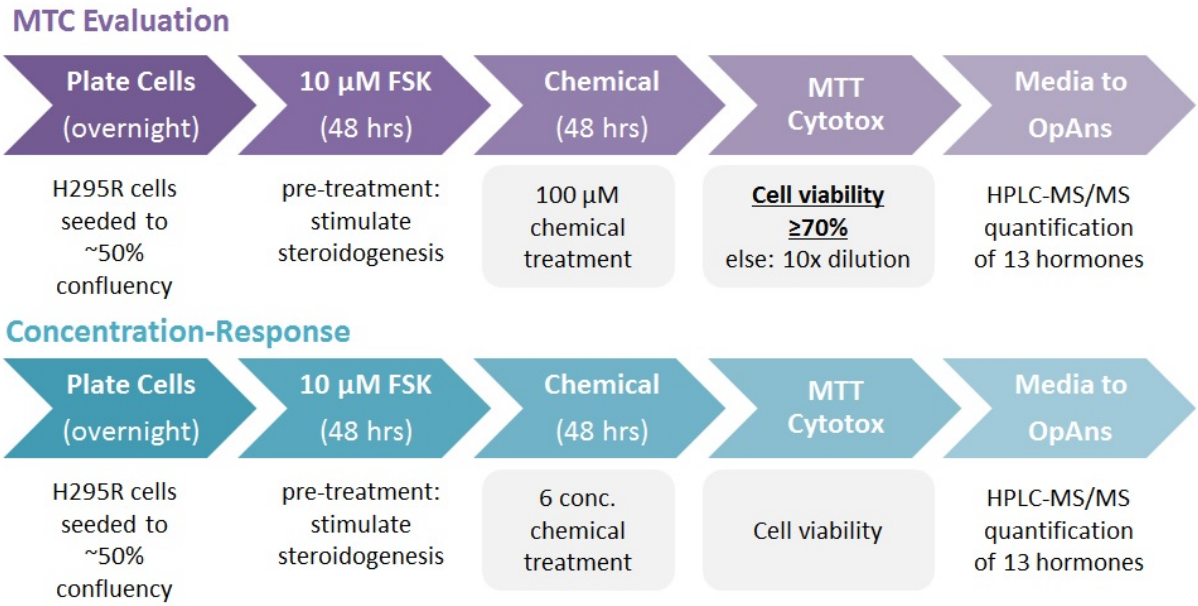

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

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Assay Publication Year:

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Method Updates / Confirmatory Studies:

None reported.

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The CeeTox H295R estrone assay was used to screen a large chemical library for changes in estrone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for estrone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of estrone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for estrone were reported as 0.03 ng/mL and 6 ng/mL, respectively, using 0.4, 1, and 16 ng/mL standards, with precision of 5.0% and accuracy of 100.4% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

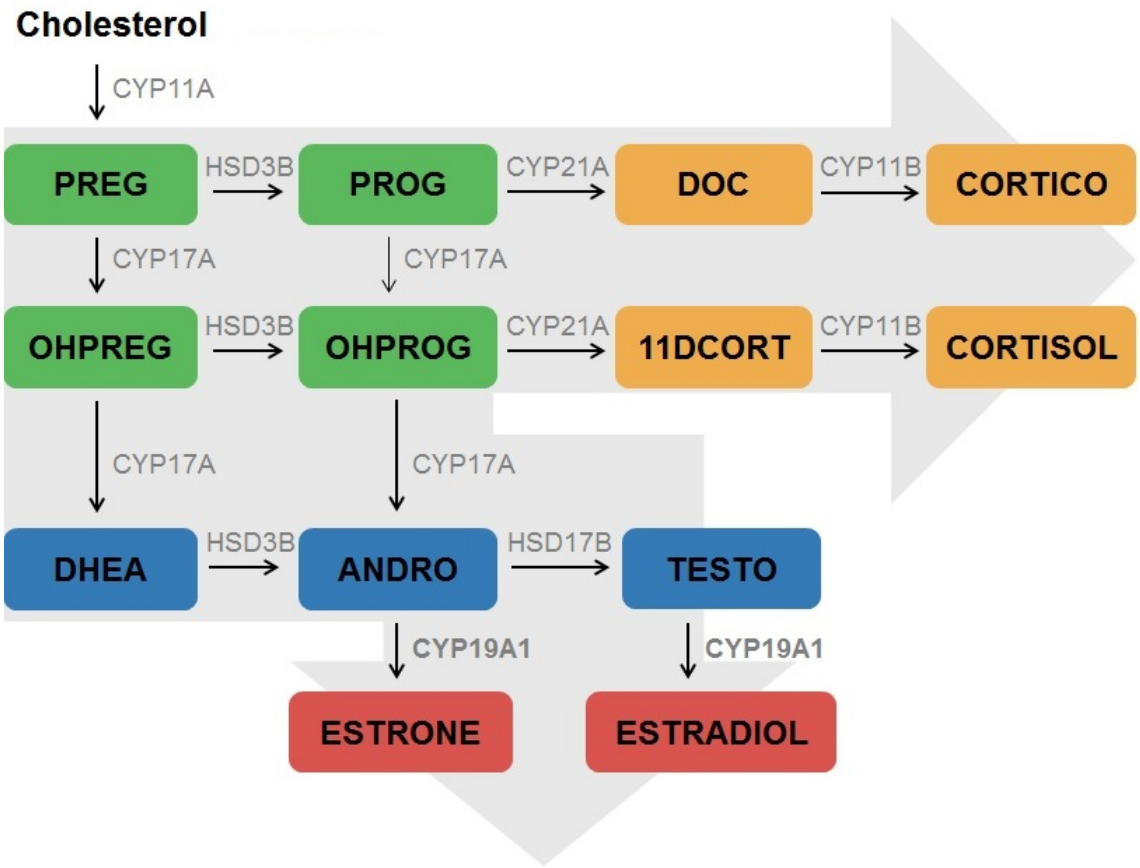

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

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USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.79 for estrone. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.76 for estrone. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 18 for estrone. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -14 for estrone. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of estrone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_ESTRONE_dn readout data was analyzed in the negative (loss of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in estrone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Estrone inhibition was determined based on a chemical fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the estrone assay this created a 1.75 fold-change cut-off); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up

CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For estrone, the $r^2 = 0.65$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

13 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_ESTRONE_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Estrone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

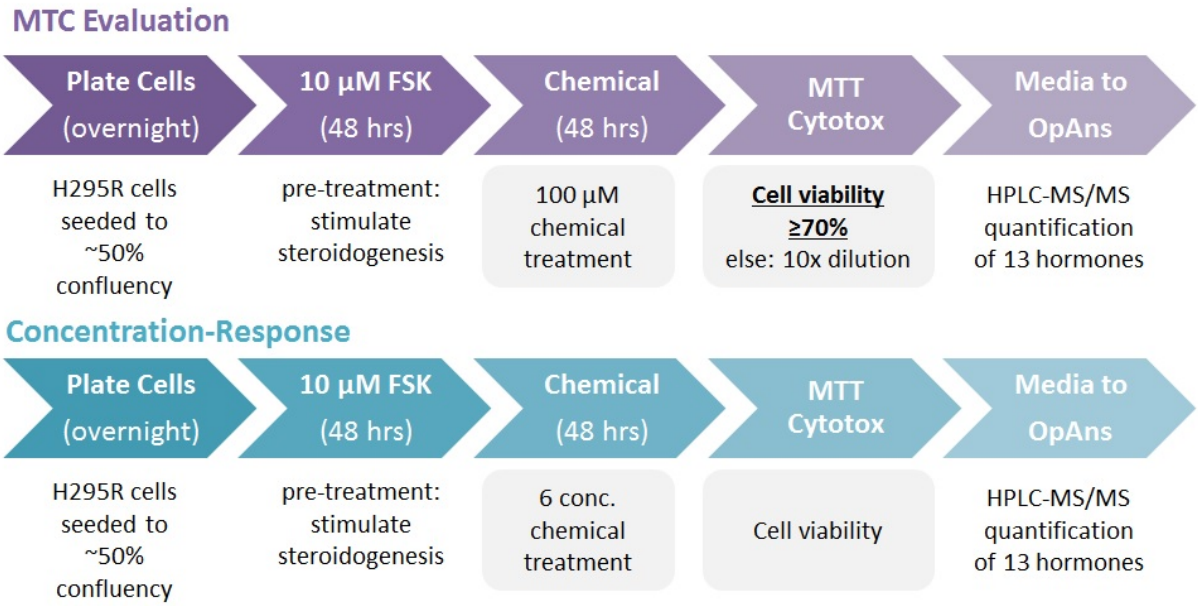

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R estrone assay was used to screen a large chemical library for changes in estrone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for estrone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of estrone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for estrone were reported as 0.03 ng/mL and 6 ng/mL, respectively, using 0.4, 1, and 16 ng/mL standards, with precision of 5.0% and accuracy of 100.4% (Karmaus *et al.*, 2016, Table 1).

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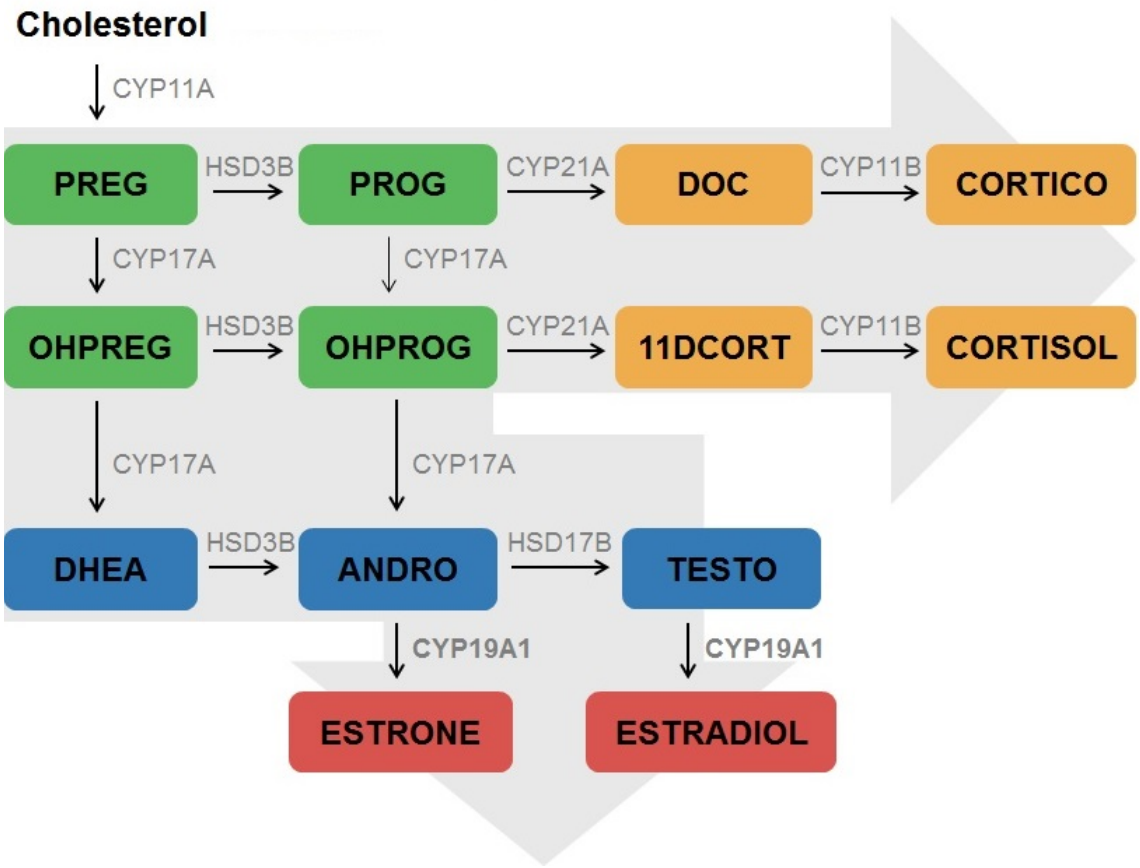

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

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3.1. Data Interpretation

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CEETOX_H295R_ESTRONE_up readout data was analyzed in the positive (gain of signal) fitting direction using the DMSO controls as the baseline signal, and was reported as log2 fold-change increase in estrone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Estrone stimulation was determined based on a chemical fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the estrone assay this created a 1.75 fold-change cut-off); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up

CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
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CEETOX_H295R_TESTO_up

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4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

13 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 892

CEETOX_H295R_OHPREG_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – 17alpha-hydroxypregnenolone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

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Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for

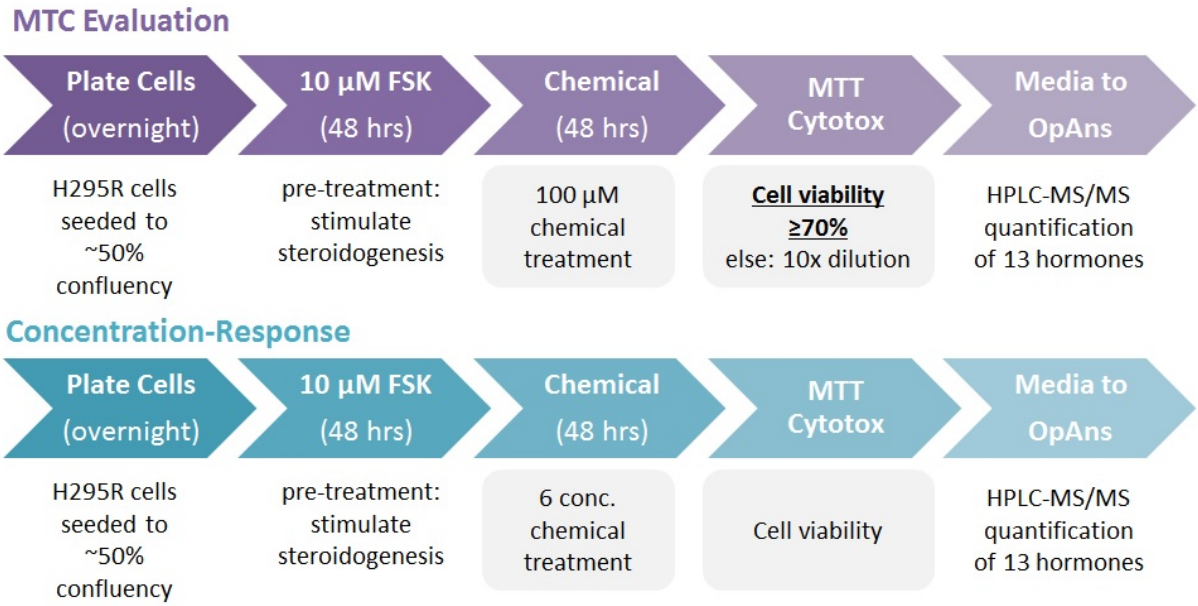
analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical



dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R 17α-hydroxypregnenolone assay was used to screen a large chemical library for changes in 17α-hydroxypregnenolone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for 17α-hydroxypregnenolone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of 17α-hydroxypregnenolone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10 µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 17α-hydroxypregnenolone were reported as 5 ng/mL and 1000 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 6.7% and accuracy of 100% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by

CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

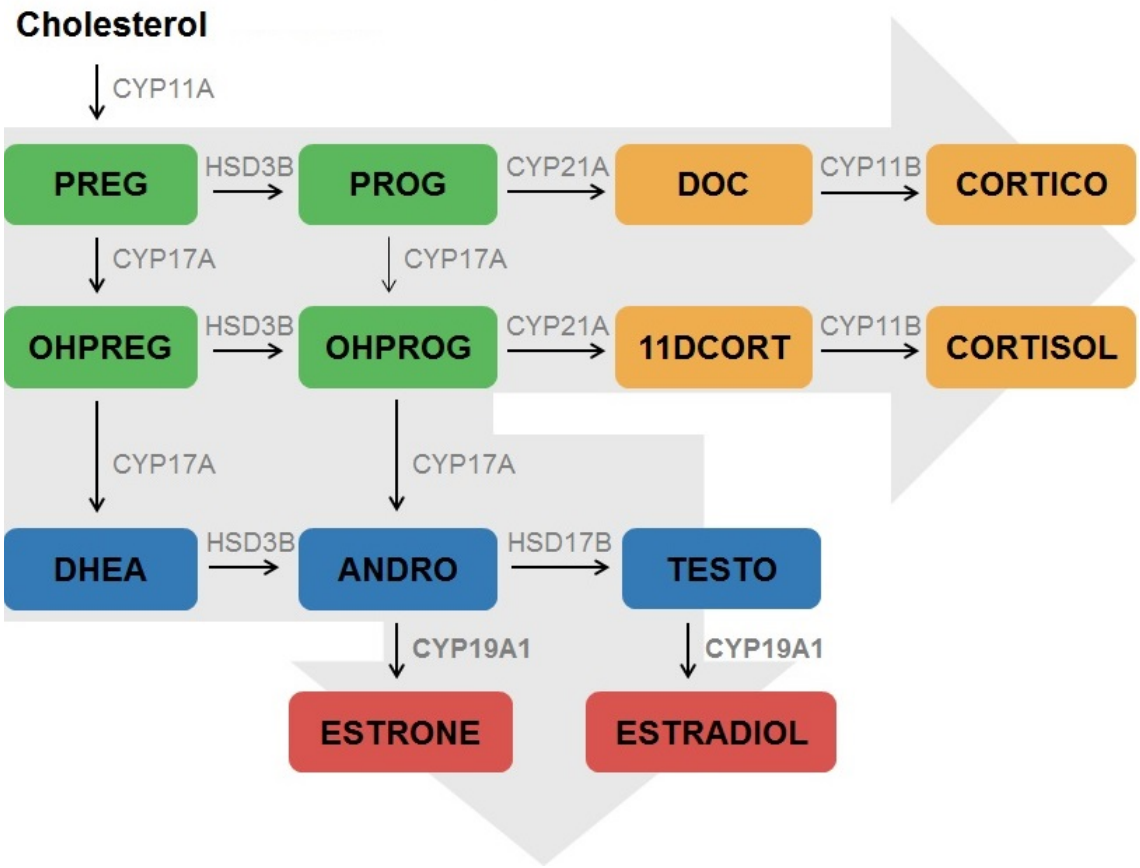


Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.81 for OHPREG. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.77 for OHPREG. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 20 for OHPREG. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of 3 for OHPREG. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of 17α-hydroxypregnenolone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_OHPREG_dn readout data was analyzed in the negative (loss of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in 17α-hydroxypregnenolone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. 17α-hydroxypregnenolone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change, which was approximately a 1.64 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn

CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
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CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For OHPREG, the $r^2 = 0.64$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
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Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

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CEETOX_H295R_OHPREG_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – 17alpha-hydroxypregnenolone Induction

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Experimental System:

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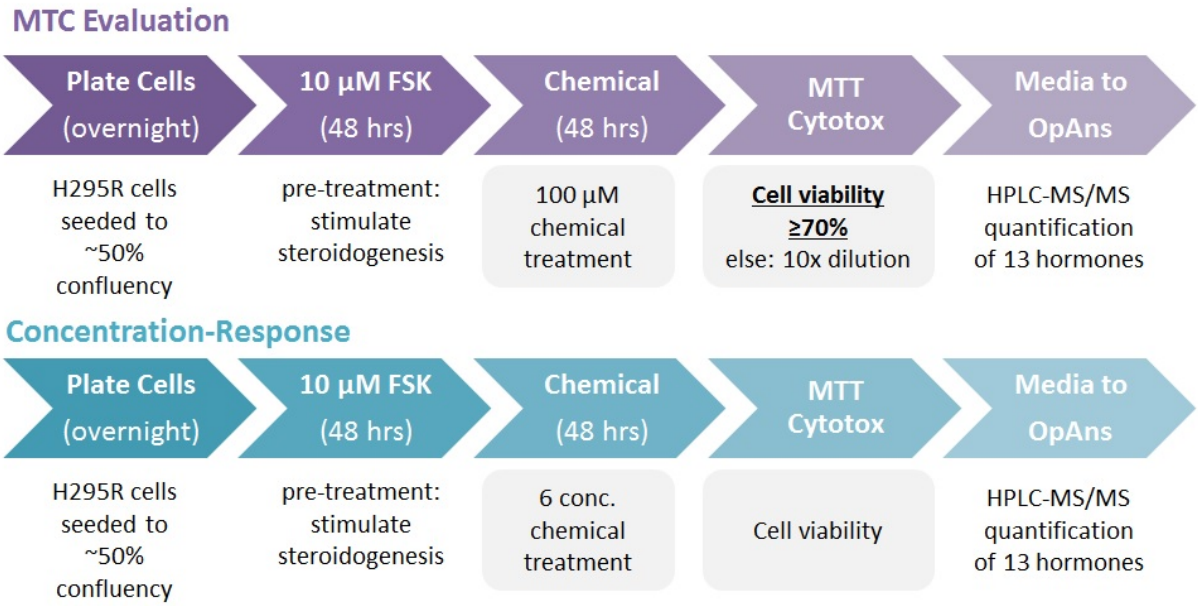

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This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R 17α-hydroxypregnenolone assay was used to screen a large chemical library for changes in 17α-hydroxypregnenolone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for 17α-hydroxypregnenolone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of 17α-hydroxypregnenolone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10 µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 17α-hydroxypregnenolone were reported as 5 ng/mL and 1000 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 6.7% and accuracy of 100% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

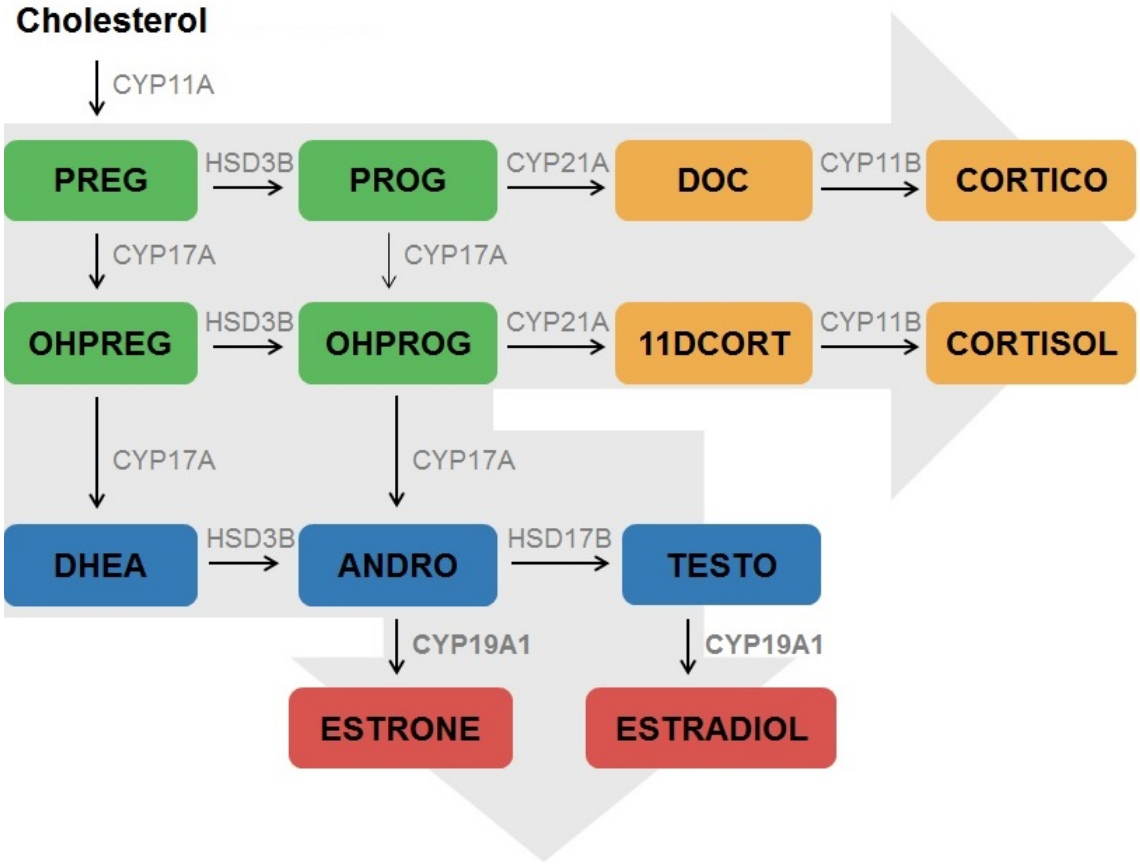


Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.81 for OHPREG. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.77 for OHPREG. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 20 for OHPREG. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of 3 for OHPREG. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of 17α-hydroxypregnenolone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_OHPREG_up readout data was analyzed in the positive (gain of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in 17α-hydroxypregnenolone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. 17α-hydroxypregnenolone stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change, which was approximately a 1.64 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than

the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For OHPREG, the $r^2 = 0.64$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.

[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)

[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)

[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.

[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion

ATCC, American Type Culture Collection

DMSO, Dimethyl Sulfoxide

HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry

MTC, Maximum Tolerated Concentration

MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide

R^2, correlation coefficient, R-squared

SSMD, strictly standardized mean difference

Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:
Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation
Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;
Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

Assay Endpoint ID: 894

CEETOX_H295R_OHPROG_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – 17alpha-hydroxyprogesterone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for

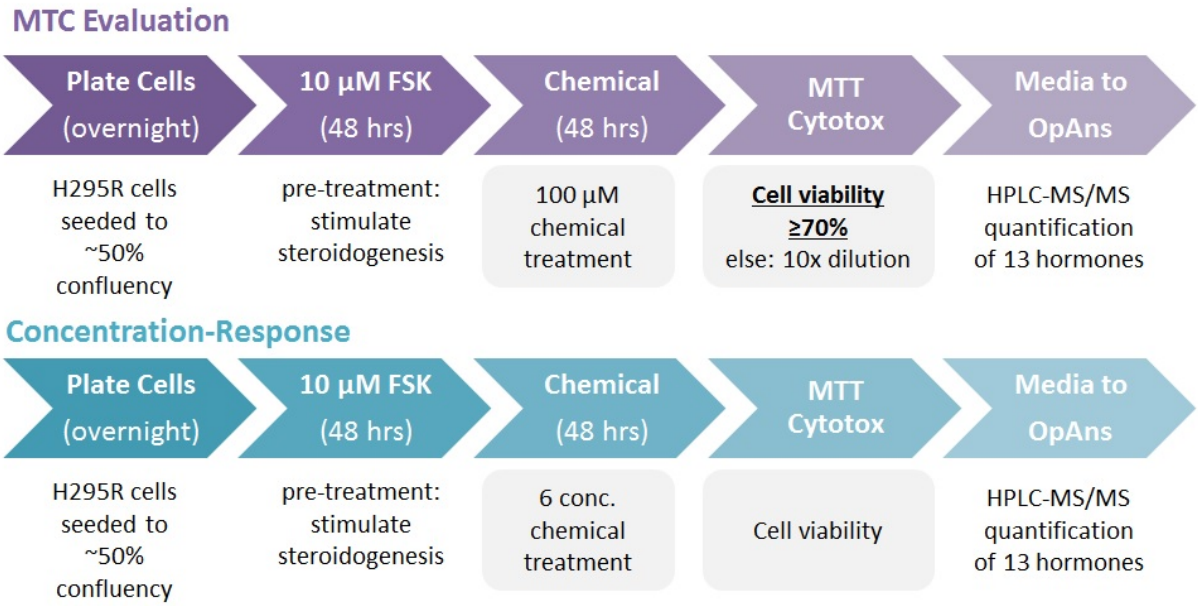
analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical



dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R 17α-hydroxyprogesterone assay was used to screen a large chemical library for changes in 17α-hydroxyprogesterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for 17α-hydroxyprogesterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of 17α-hydroxyprogesterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10 µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 17α-hydroxyprogesterone were reported as 0.2 ng/mL and 40 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 4.0% and accuracy of 99.6% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by

CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

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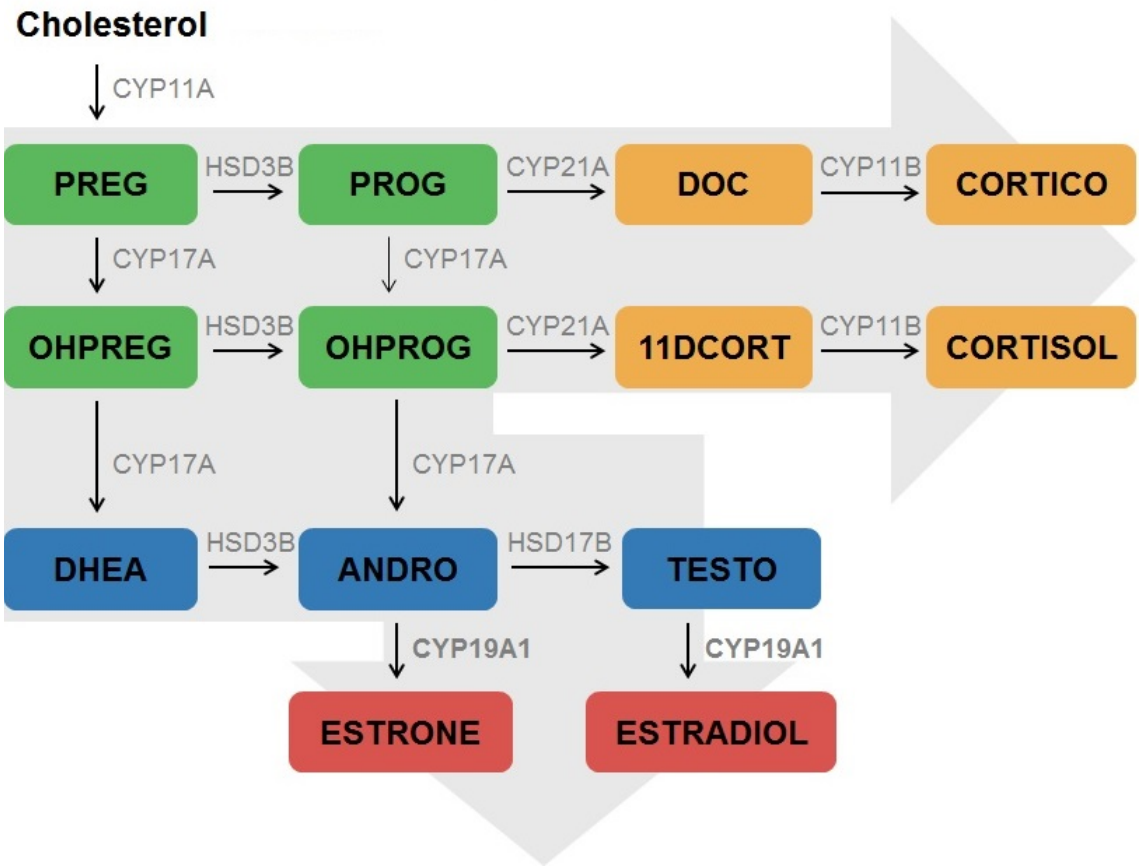

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

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3.1. Data Interpretation

Biological Response:

Decreased production of 17α-hydroxyprogesterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_OHPROG_dn readout data was analyzed in the negative (loss of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in 17α-hydroxyprogesterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. 17α-hydroxyprogesterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change; in the 17α-hydroxyprogesterone assay this was approximately a 1.87 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn

CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For OHPROG, the $r^2 = 0.71$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.

[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
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[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 895

CEETOX_H295R_OHPROG_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – 17alpha-hydroxyprogesterone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

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Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for

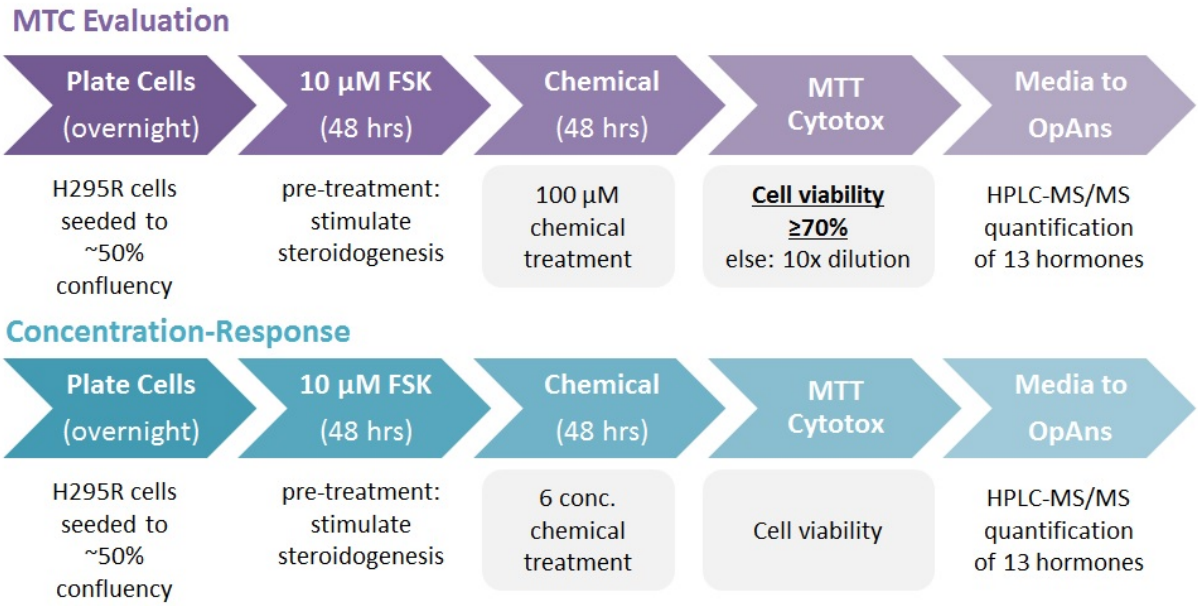
analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical



dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R 17α-hydroxyprogesterone assay was used to screen a large chemical library for changes in 17α-hydroxyprogesterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for 17α-hydroxyprogesterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of 17α-hydroxyprogesterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10 µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for 17α-hydroxyprogesterone were reported as 0.2 ng/mL and 40 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 4.0% and accuracy of 99.6% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

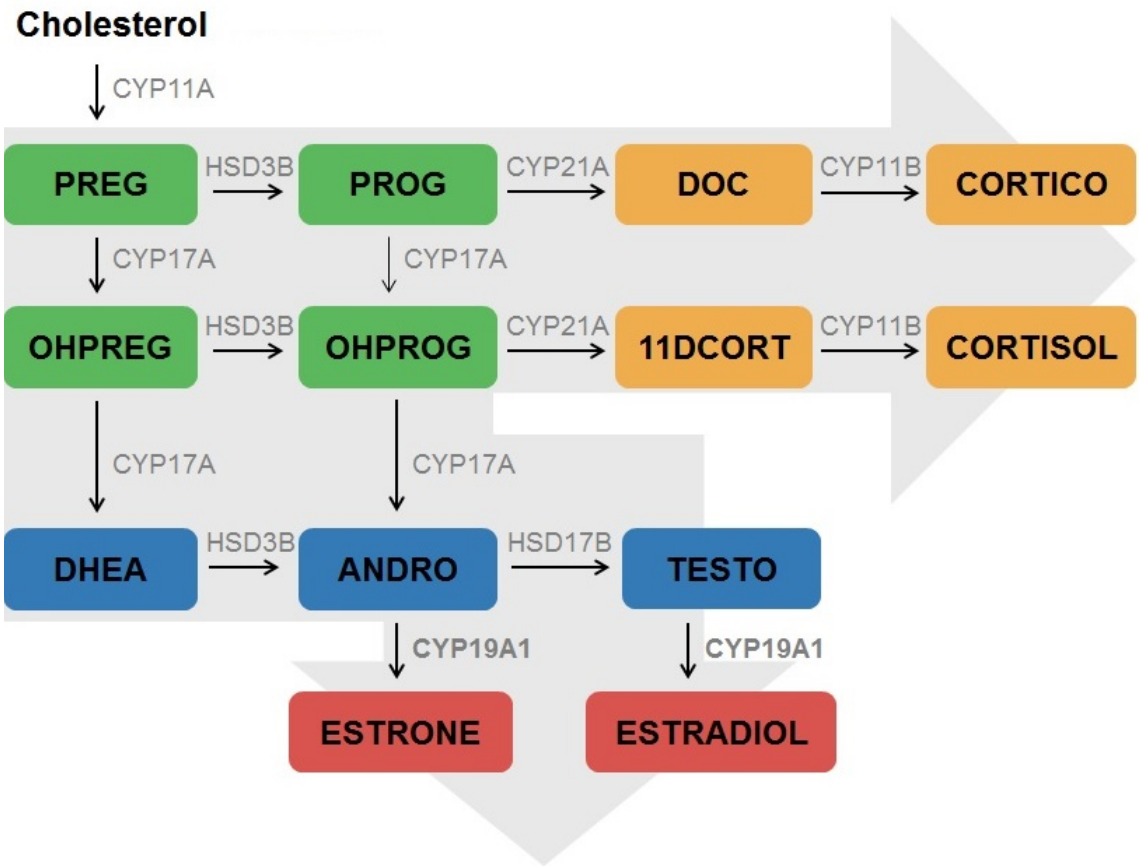


Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.62 for OHPROG. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0 for OHPROG. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 10 for OHPROG. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -2 for OHPROG. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of 17α-hydroxyprogesterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_OHPROG_up readout data was analyzed in the positive (gain of signal) fitting direction uisng DMSO controls as the baseline signal, and was reported as log2 fold-change increase in 17α-hydroxyprogesterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. 17α-hydroxyprogesterone stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the 17α-hydroxyprogesterone assay this was approximately a 1.87 fold-change cut-off); the modeled top (modl_tp) of the curve was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn

CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
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[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.

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Research Triangle Park, NC 27711
919-541-4219

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Date of Revisions:

Author of Revisions:

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CEETOX_H295R_PREG_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Pregnenolone Inhibition

1. Assay Descriptions

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H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

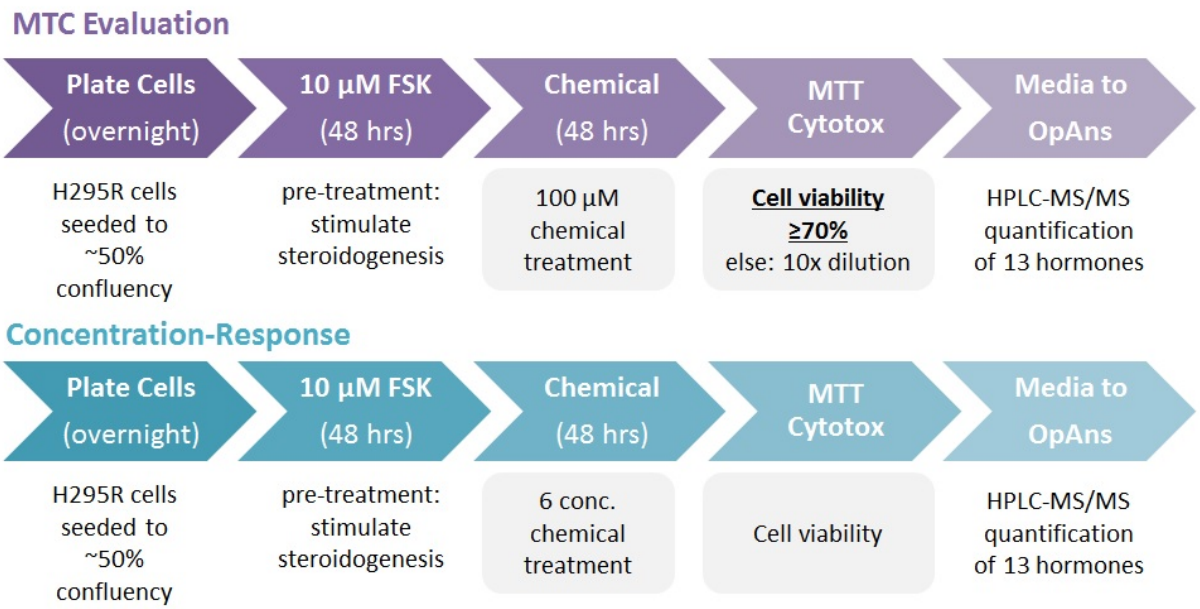

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

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Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Pregnenolone assay was used to screen a large chemical library for changes in pregnenolone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, media was subject to MBTE and dansyl chloride derivatization for pregnenolone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of pregnenolone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for pregnenolone were reported as 2 ng/mL and 400 ng/mL, respectively, using 20, 50, and 800 ng/mL standards, with precision of 10.0% and accuracy of 100.9% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

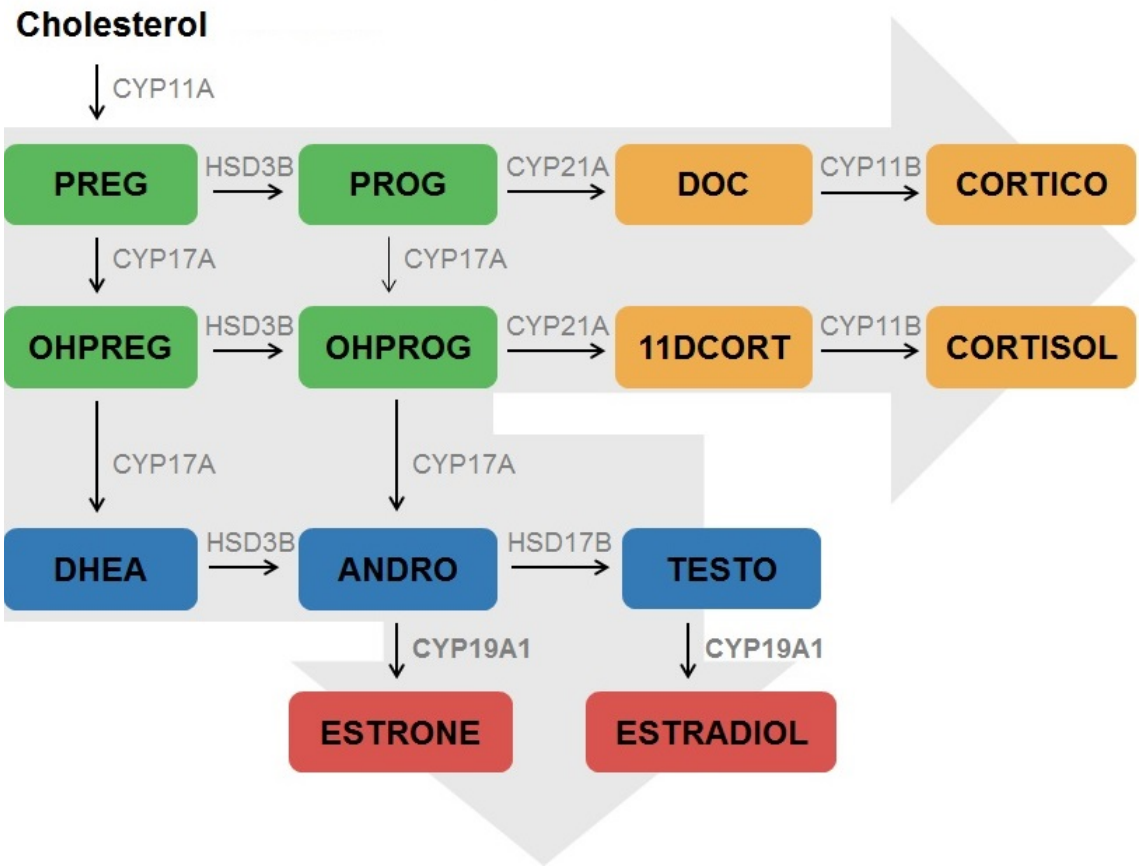

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18. SSMD-prochloraz ranged from -27-7. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of pregnenolone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_PREG_dn readout data was analyzed in the negative (loss of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in pregnenolone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Pregnenolone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up

CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_PREG_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Pregnenolone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

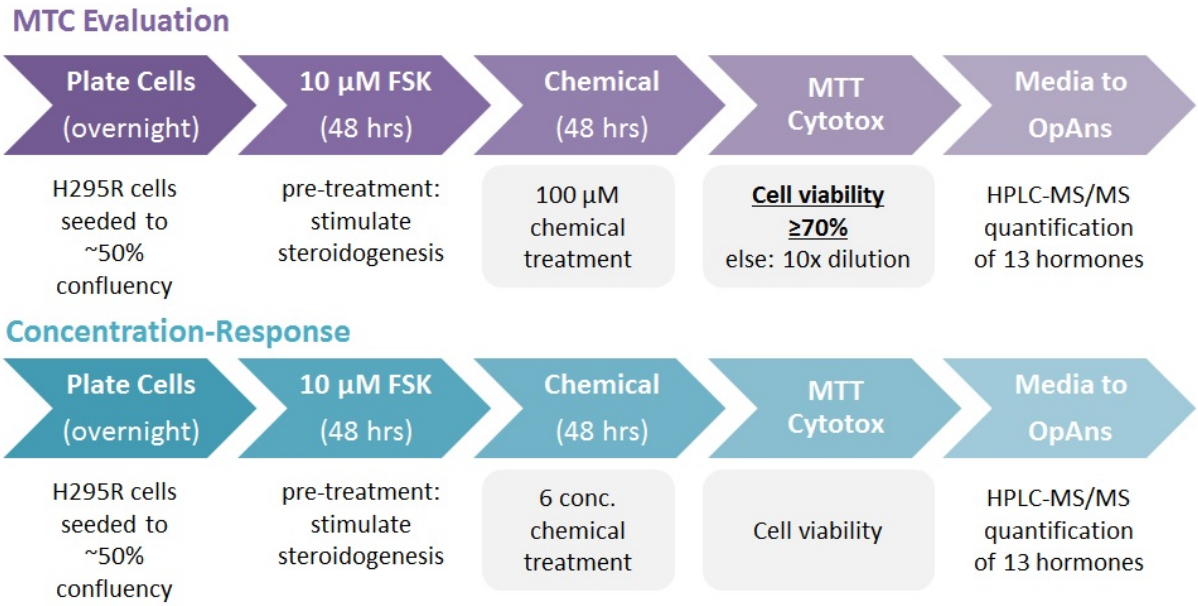

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

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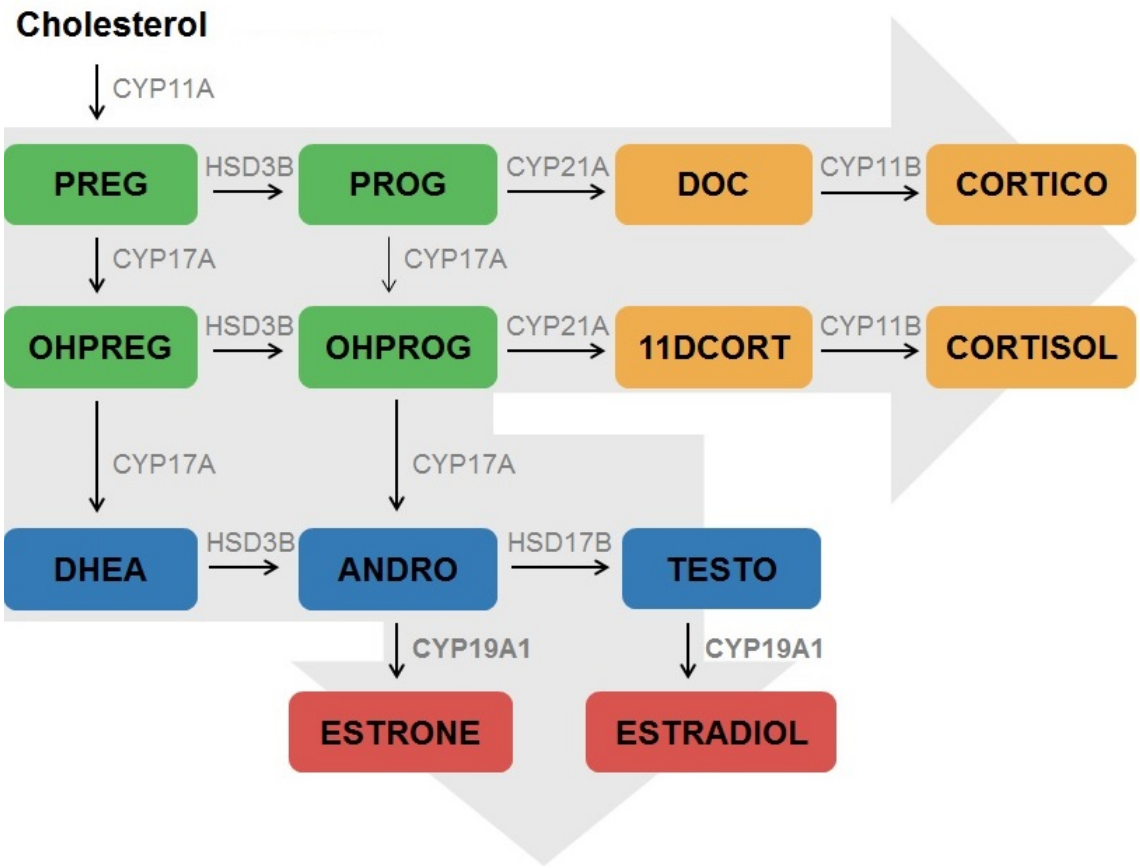

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

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Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.76 for 11DCORT. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.88 for 11DCORT. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18. SSMD-prochloraz ranged from -27-7. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7|demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of pregnenolone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_PROG_up readout data was analyzed in the positive (gain of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in pregnenolone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were log_2-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Pregnenolone stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in log_2 fold-change); the modeled top of the curve (modl_tp) was above the established response cutoff; and the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn

CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
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4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_PROG_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Progesterone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

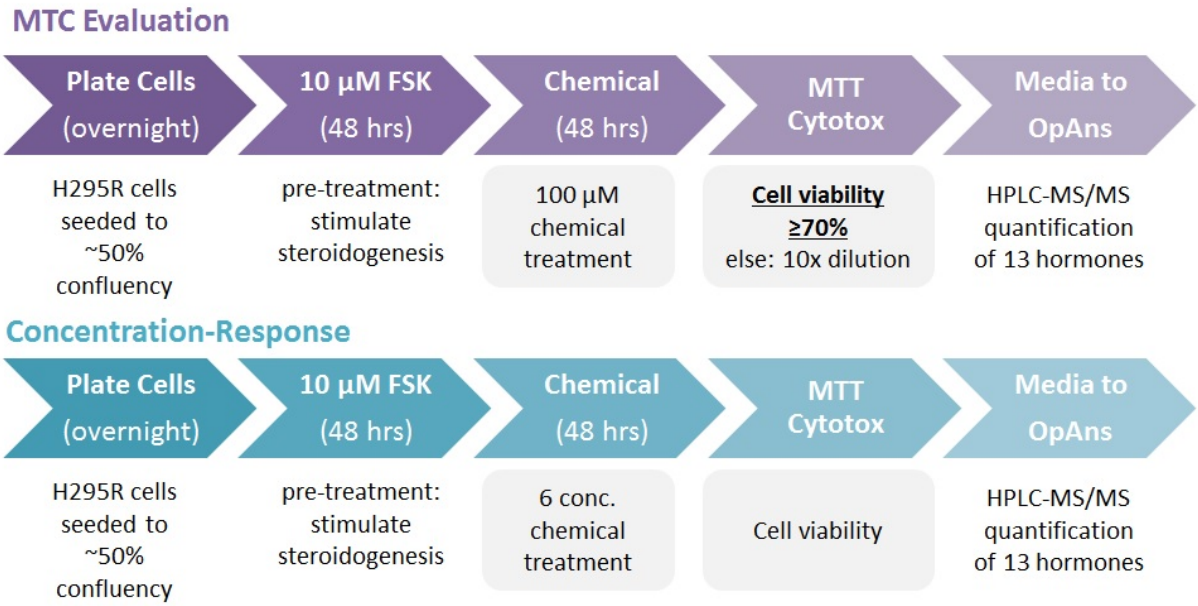

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R progesterone assay was used to screen a large chemical library for changes in progesterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for progesterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of progesterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for progesterone were reported as 0.2 ng/mL and 40 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 3.3% and accuracy of 98.1% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

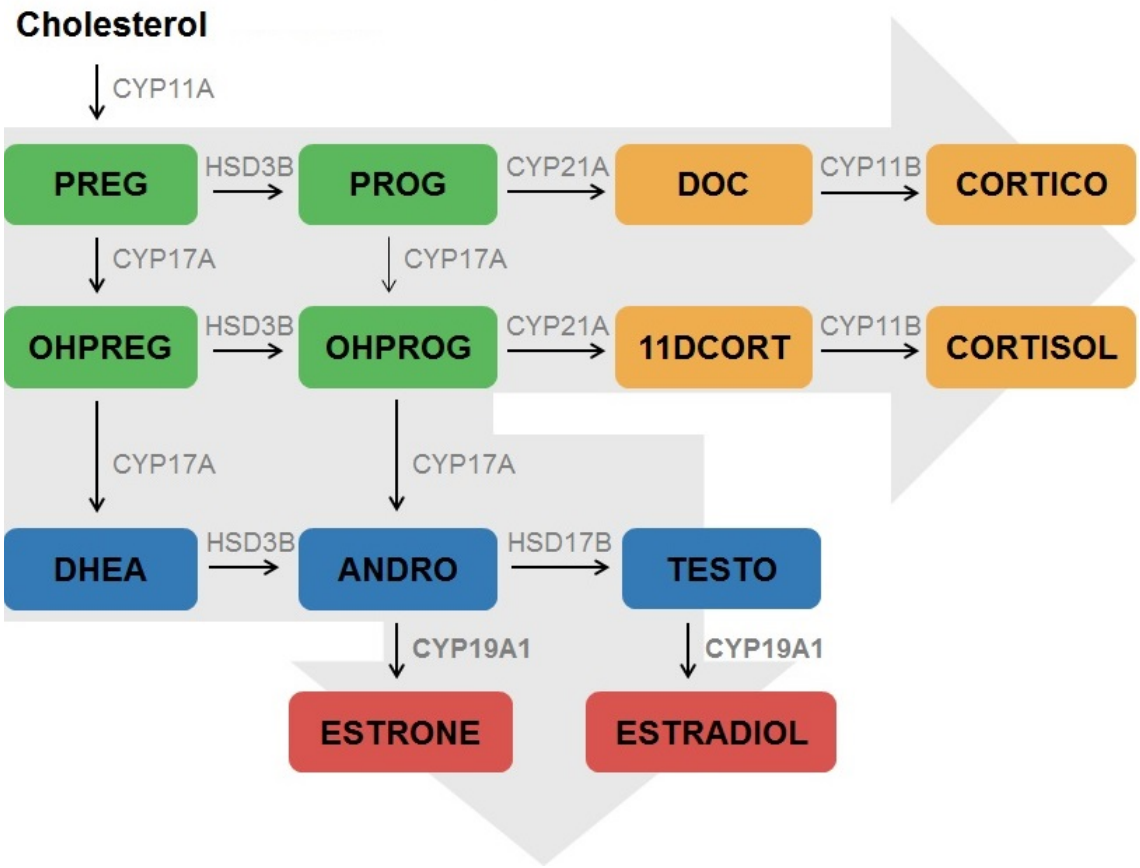

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.0 for progesterone. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.85 for progesterone. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 1 for progesterone. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of 21 for progesterone. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of progesterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_PROG_dn readout data was analyzed in the negative (loss of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in progesterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Progesterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the progesterone assay this was approximately a 1.97 fold-change cut-off); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_PROG_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Progesterone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

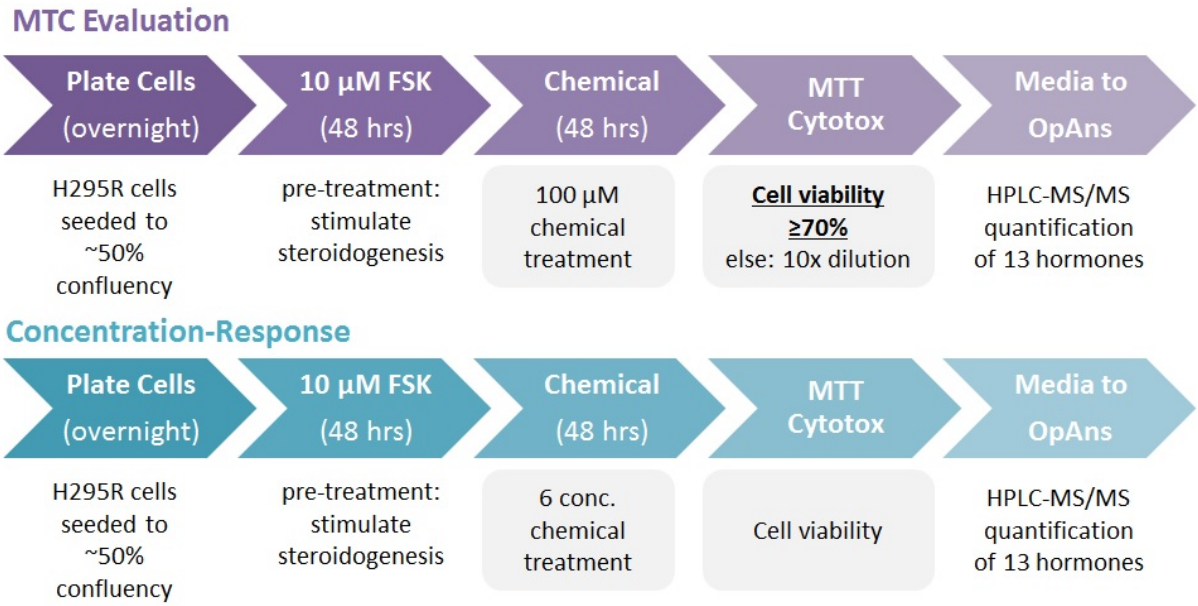

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R Progesterone assay was used to screen a large chemical library for changes in progesterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for progesterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of progesterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for progesterone were reported as 0.2 ng/mL and 40 ng/mL, respectively, using 4, 10, and 160 ng/mL standards, with precision of 3.3% and accuracy of 98.1% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

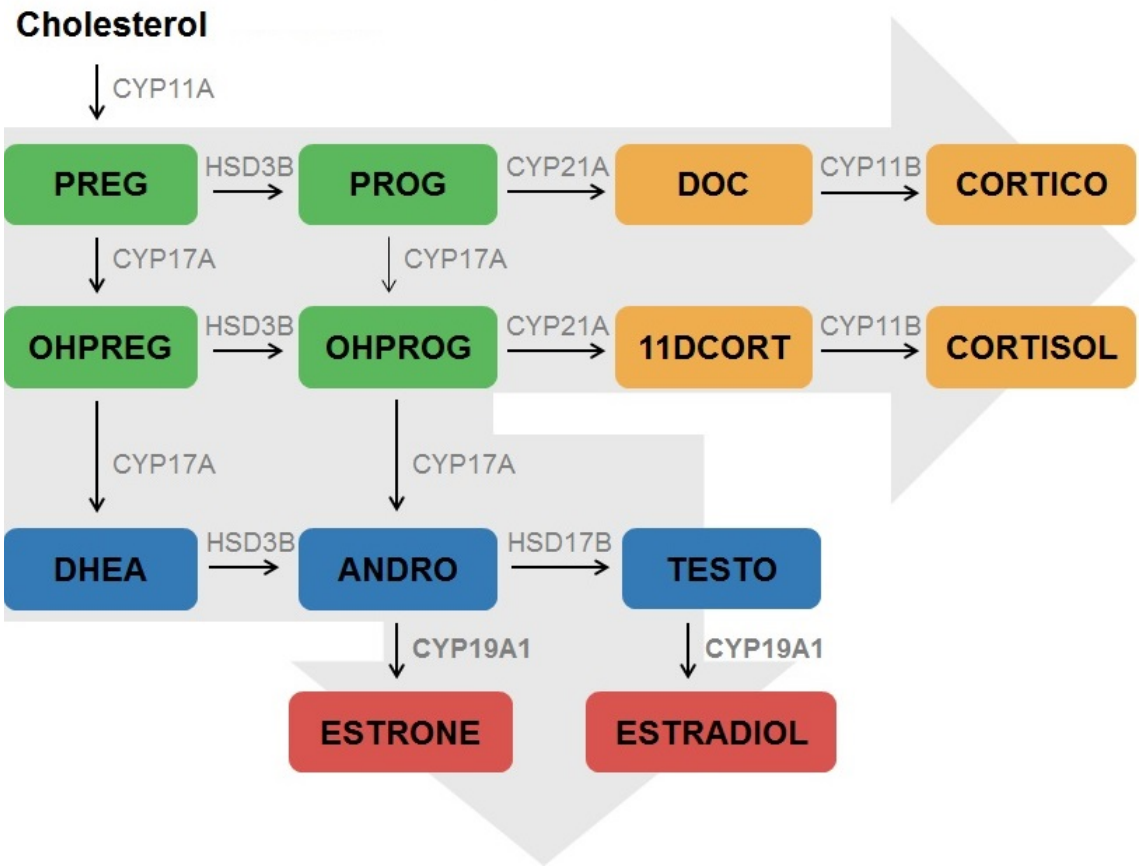

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.0 for progesterone. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.85 for progesterone. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 1 for progesterone. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of 21 for progesterone. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Increased production of progesterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_PROG_up readout data was analyzed in the positive (gain of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in progesterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Progesterone stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the progesterone assay this was approximately a 1.97 fold-change cut-off); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn

CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For progesterone, the $r^2 = 0.79$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.

[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_TESTO_dn

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Testosterone Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

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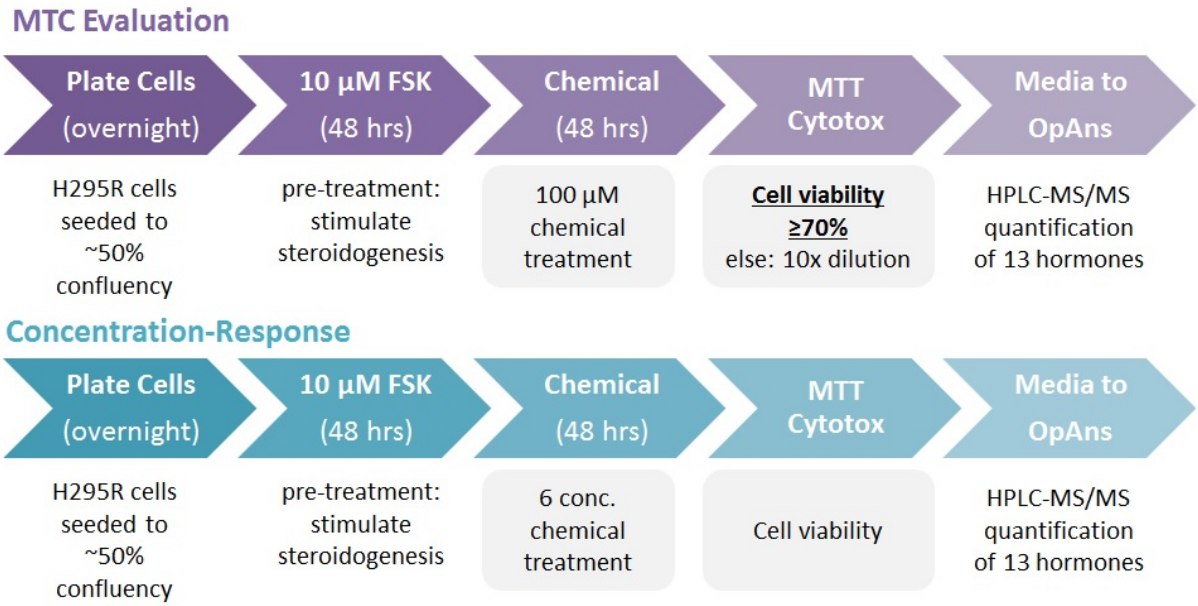

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

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Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
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Fax: 1-617-812-0712
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Assay Publication Year:

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Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R testosterone assay was used to screen a large chemical library for changes in testosterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for testosterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of testosterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for testosterone were reported as 0.1 ng/mL and 20 ng/mL, respectively, using 0.4, 1, and 16 ng/mL standards, with precision of 5.7% and accuracy of 100.7% (Karmaus *et al.*, 2016, Table 1).

Scientific Principles:

The steroidogenic pathway is a series of hydroxylation and dehydrogenation steps carried out by CYP450 and hydroxysteroid dehydrogenase enzymes. Environmentally-relevant chemicals can elicit endocrine disruption by altering steroid hormone biosynthesis and metabolism (steroidogenesis), resulting in adverse reproductive and developmental effects. The ToxCast HTS program adapted the OECD-validated H295R steroidogenesis assay using human adrenocortical carcinoma cells (TG No. 456; which established performance-based testing guidelines for the quantification of two steroid hormones, testosterone and estradiol) to quantitatively assess the concentration-dependent effects of chemicals on 13 steroid hormones in 4 hormone classes including progestagens, androgens, estrogens and glucocorticoids.

To identify xenobiotics with the capacity to disrupt the steroid hormone biosynthetic pathway, this assay quantitatively assesses changes in steroid hormone levels. The CeeTox Steroidogenesis assay also demonstrates a novel implementation of H295R cells as a high-throughput and multiplexed screening platform for screening a diverse chemical library for xenobiotic interference with steroidogenesis.

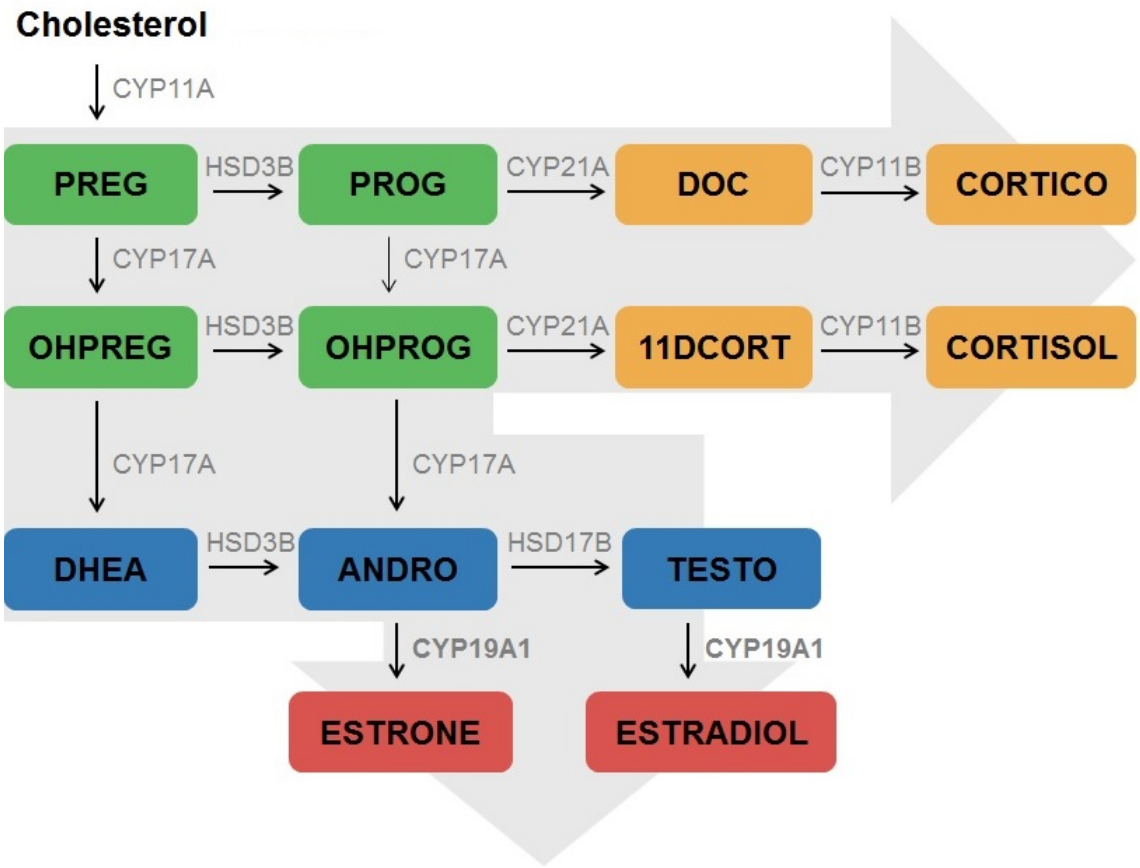

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

OECD (2011). *Test No. 456 H295R Steroidogenesis Assay.* OECD Publishing, Paris

USEPA (2011). *Steroidogenesis (Human Cell Line – H295R)* OCSPP Guideline 890.1550. Standard Evaluation Procedure (SEP) Endocrine Disruptor Screening Program.

Assay Quality Statistics:

Robust Z-prime (Z') was calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and positive control (forskolin and prochloraz) wells. The Z' for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. Z'-forskolin ranged from 0.57-0.81, with a Z'-forskolin of 0.57 for testosterone. Z'-prochloraz ranged from 0.51-0.88, with a Z'-prochloraz of 0.75 for testosterone. Z' values ranging between 0.5 and 1 demonstrate that an assay signal can be reliably identified from variability around the baseline control.

The strictly standardized mean difference (SSMD) values were calculated per hormone analyte using the median of the raw response values across plates for the baseline control (DMSO) and

positive control (forskolin and prochloraz) wells. The SSMD for forskolin and prochloraz are presented in Table 2 of Karmaus et al. 2016, with separate values presented for each hormone to reflect the different positive control wells present on the plate included in the analysis of specific hormones. SSMD-forskolin ranged from 1-18, with a SSMD-forskolin of 8 for testosterone. SSMD-prochloraz ranged from -27-7, with a SSMD-prochloraz of -13 for testosterone. SSMD is a measure of effect size and a means of demonstrating directionality, i.e. differences between positive and negative control response; values ≥ |7| demonstrate excellent dynamic range and robust responses with the positive and negative controls.

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Decreased production of testosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_TESTO_dn readout data was analyzed in the negative (loss of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in testosterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Testosterone inhibition was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the testosterone assay this created a 2.05 fold-change cut-off); if the modeled top of the curve (modl_tp) was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publically available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

CEETOX_H295R_11DCORT_dn
CEETOX_H295R_11DCORT_up
CEETOX_H295R_ANDR_dn
CEETOX_H295R_ANDR_up
CEETOX_H295R_CORTIC_dn
CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up

CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_up

3.2. Assay Performance

Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median log_2-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For testosterone, the $r^2 = 0.57$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.
[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)

[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

13 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

CEETOX_H295R_TESTO_up

Assay Title: CeeTox H295R High-throughput Steroidogenesis Assay – Testosterone Induction

1. Assay Descriptions

1.1. Overview

Assay Summary:

Endocrine disrupting chemicals have largely been studied by examining effects on nuclear receptor (primarily estrogen and androgen receptor) ligand binding or signaling pathways, but environmental chemicals can also disrupt endocrine systems by interfering with steroid hormone biosynthesis and metabolism (steroidogenesis). The CeeTox H295R steroidogenesis assays evaluate a human-derived, steroidogenically-competent adenocarcinoma cell line (H295R) in high-throughput (96-well) format to detect substances with the potential to disrupt steroid hormone production. To detect interference with steroidogenesis, this assay quantified 13 different steroid hormones using high-throughput HPLC-MS/MS. The assay is a modified version of the OECD H295R test guideline (OECD TG 456). Briefly, cells were seeded into 96-well plates and after overnight incubation were pre-treated with 10 µM forskolin for 48 hours to stimulate steroidogenesis. Following the pre-treatment, medium was replaced with test chemical for 48 hours. Following chemical exposure, medium was removed for steroid hormone analysis, and cells were subjected to the MTT assay as an indicator of cell viability. This assay involved a three-tiered screening strategy. The initial stage was designed to identify a maximum tolerated concentration (MTC) of test chemical in H295R cells. Briefly, chemicals were evaluated at up to 100 µM (solubility permitting) using the MTT assay, in order to determine the maximum concentration that maintained ≥70% cell viability. Following MTC determination, each chemical was screened for effects on steroidogenesis by quantifying changes in hormone levels at the MTC (single test concentration). Chemicals selected for follow-up were evaluated in dose-response to establish whether changes in hormone levels were concentration-dependent. In an initial screening exercise, chemicals were selected for concentration-response follow-up if at least 4 different hormones were significantly affected when screened at the MTC. Concentration-response experiments were conducted using six concentrations, serially diluted down from the MTC at half-log increments.

1.2. Assay Definition

Assay Throughput:

Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times (maximum passage of 10). Cells were seeded into 96-well plates at 50-60% confluency and allowed to adhere overnight. Prior to test chemical exposure, cells were pre-stimulated with forskolin for 48 hours, and chemical exposures were subsequently conducted for 48 hours in forskolin-free media. Following concentration-response and MTC assays, MTT cytotoxicity assays were conducted. The media samples were stored at -80 °C prior to HPLC-MS/MS quantification of steroid hormones.

Experimental System:

H295R is an immortalized cell line derived from an adrenocortical carcinoma isolated in 1980 from a 48-year-old African-American female patient. H295R cells express genes that encode for all the key enzymes involved in steroidogenesis [1, 2]. H295R cells have the physiological characteristics of zonally undifferentiated human fetal adrenal cells. H295R cells represent a unique in vitro system, as the cells retain the ability to produce many of the steroid hormones found in the adult adrenal cortex and the gonads (with the exception of dihydrotestosterone, DHT), which allows for analysis of xenobiotic effects on both corticosteroid synthesis and the production of sex steroid hormones, including androgens and estrogens.

Xenobiotic Biotransformation Potential:

H295R cell line expresses CYPs 1A1, 11A, 17, 19, 21, 1B1 and 11B1, which are differentially induced by endocrine-disrupting chemicals [3, 4].

Basic Procedure:

Cell culture and media preparation procedures were conducted in accordance with OECD Test No. 456 guidelines [1], with minor modifications. H295R cells (ATCC CRL-2128) were expanded for 5 passages and frozen in batches in liquid nitrogen. Prior to the steroidogenesis assay, H295R cells were thawed and passed at least 4 times. The maximum passage was 10. Cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 5ml/L ITS+ Premix (BD Bioscience) and 12.5 ml/L Nu-Serum (BD Bioscience). After seeding cells into 96-well plates at 50-60% confluency, cells were allowed to adhere overnight. Prior to chemical testing, culture medium was replaced with 175 µL medium containing 10 µM forskolin to stimulate steroidogenesis for 48 hours. Following pre-stimulation, forskolin medium was replaced with medium containing test chemical solubilized in DMSO (ensuring a final concentration of 0.1% DMSO). Test chemicals were incubated for 48 hours, then the medium was removed and split into 2 vials containing approximately 75µL each, and stored at -80 °C prior to hormone analysis. For steroid quantification, media samples were shipped to OpAns, LLC (Durham, NC) on dry ice, and samples were thawed to room temperature prior to liquid-liquid extraction. Steroid hormones were extracted from media samples using methyl tert-butyl ether (MTBE). An extra derivatization with dansyl chloride was included for estrogen (estrone and estradiol) detection only. Steroid hormones were separated by HPLC, eluted using a reverse phase C18 gradient with electrospray positive ionization, followed by quantification by tandem mass spectrometry (MS/MS). Data were acquired on a MassHunter Workstation Acquisition version B03.01 (Agilent Technologies, Inc.) and processed using MassHunter Quantitative Analysis for QQQ. Accuracy was determined for each hormone analyte from 3 standards to determine upper and lower limits of quantification (ULOQ and LLOQ, respectively) using a 7-point standard curve. Precision and accuracy of the extraction and quantification methods were calculated as the percent relative standard deviation (%RSD) of the spiked standards and percent spiked standard recovered, respectively. The goal was to achieve 100% accuracy (i.e., recover all spiked-in standard at quantification with minimal loss during run time) and good precision (i.e., have %RSD <15% to ensure reproducibility). Test medium was removed following chemical exposure, and cell viability was evaluated in the same wells by MTT [5]. MTT procedures were as follows: after removal of test medium, 500 µl of 0.5 mg/ml of 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide solution was added to the cells. Following a 4-hour incubation at 37 °C and 5% CO_2 to allow formazan-crystal formation, the MTT solution was removed and blue formazan salt crystals were solubilized using 500µl anhydrous isopropanol with shaking for 20 minutes. Absorbance was read at 570 and 650nm using a Packard Fusion microplate reader. Background correction of absorbance units was used to determine percent change relative to controls. All assay plates contained multiple control wells including four 10µM forskolin replicates to control for hormone stimulation, four 3µM procholaz replicates to control for hormone inhibition and 4 digitonin replicates to control for cell death. Cell viability, as indicated by the MTT assay, was used to establish maximum tolerated concentration (MTC) initially using a nominal concentration of 100µM (where feasible within solubility limits of the specific test chemical) and targeting cell viability ≥70%. When a test chemical reduced cell viability to 20% - 70%, the test chemical was diluted 10-fold and re-evaluated, but if cell viability was <20%, then the test chemical was diluted 100-fold and re-evaluated. Test chemical dilutions were made until cell viability was ≥70%. MTT assays were also conducted for duplicates of all concentrations of chemicals tested in concentration-response studies.

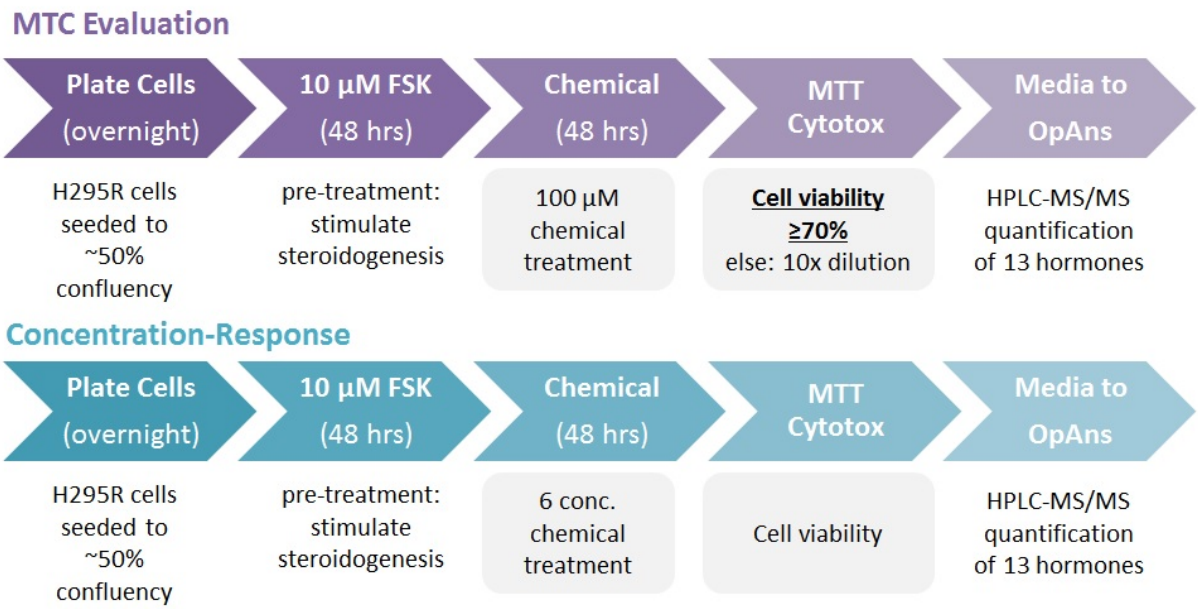

Figure 1. Schematic representation of general testing protocol for CeeTox Steroidogenesis assays. (Adapted from Karmaus et al. 2016.)

Proprietary Elements:

This assay is not proprietary; it is a modification of the existing OECD H295R *in vitro* steroidogenesis assay validated in 2011 (Test Guideline No. 456, [1]). NCI-H295R cells (ATCC CRL-2128) are commercially available from American Type Culture Collection with signed Material Transfer Agreement.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to interfere with endogenous steroidogenesis, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Cyprotex (Formerly CeeTox, Inc. in Kalamazoo, MI)
313 Pleasant St.
Watertown, MA 02472
1-888-297-7683
Fax: 1-617-812-0712
enquiries@cyprotex.com

Assay Publication Year:

2016

Assay Publication:

Karmaus AL, Toole CM, Filer DL, Lewis KC, Martin MT. 2016. High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells. Toxicological Sciences 150:323-332.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The CeeTox H295R testosterone assay was used to screen a large chemical library for changes in testosterone levels resulting from interference with steroidogenesis in H295R human adrenocortical carcinoma cells. For an initial screening application, a three-tiered screening approach was employed. The first stage involved establishing the maximum tolerated concentration (MTC) for each test chemical, respectively, which was identified using the MTT assay as an indicator of cell viability. Once MTC was determined, cells were treated with that concentration to evaluate steroid hormone changes at the MTC. In this single concentration "MTC screening" assay, any response ≥ |1.5-fold| relative to DMSO controls was considered to represent significant effects on hormone production. Following the MTC single concentration screen, those chemicals with significant effects on at least 4 hormones were considered active and subsequently assessed in concentration-response format using a 6-concentration series. After chemical treatment, which consists of 48hr forskolin pre-stimulation followed by 48hr chemical treatment, medium was subjected to MBTE and dansyl chloride derivatization for testosterone quantification. HPLC-MS/MS spectra were resolved and the absolute quantity of testosterone from the medium sample was reported in ng/mL. Chemicals were tested in duplicate, and each plate included duplicated controls for stimulation (10µM forskolin) and inhibition (3 µM procholaz) of steroidogenesis. 4 replicates per plate of DMSO-only solvent controls served as neutral controls. The LLOQ and LLOD for testosterone were reported as 0.1 ng/mL and 20 ng/mL, respectively, using 0.4, 1, and 16 ng/mL standards, with precision of 5.7% and accuracy of 100.7% (Karmaus *et al.*, 2016, Table 1).

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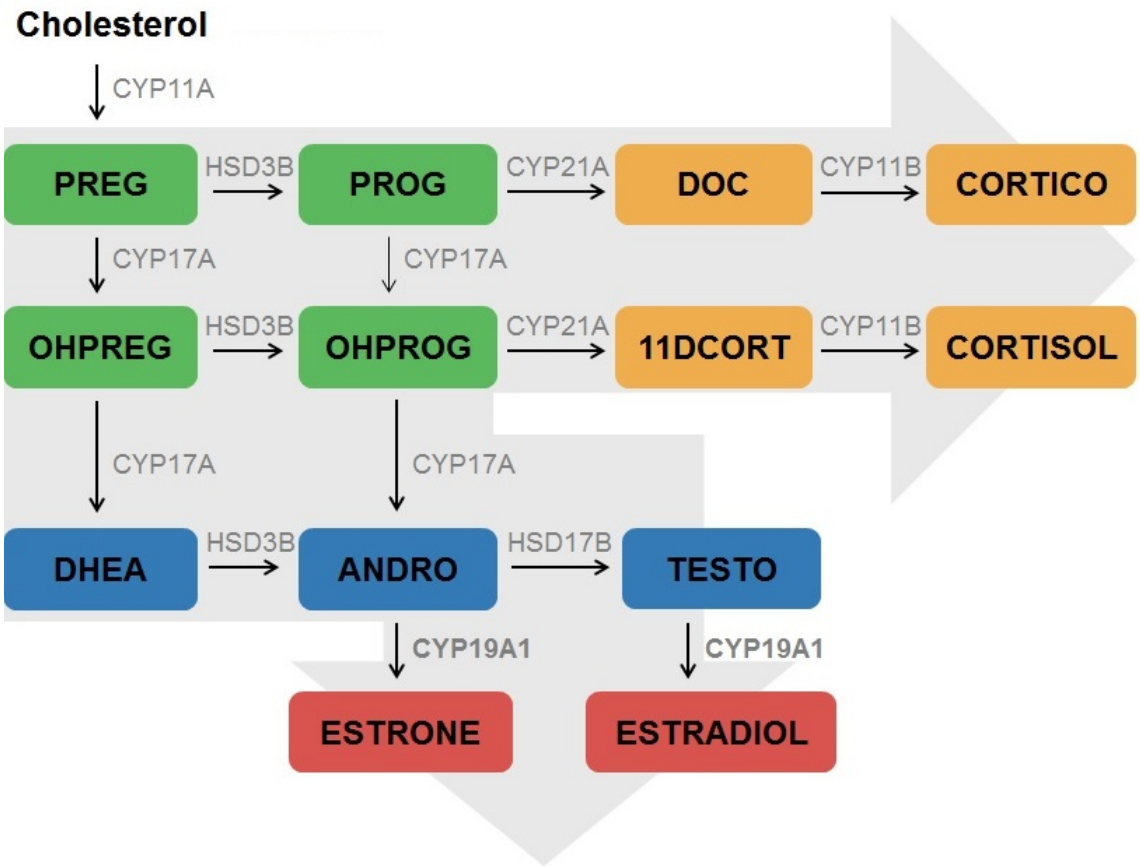

Figure 2. Subset of the steroidogenesis pathway targeted by the CeeTox H295R Steroidogenesis assays. (Modified from Karmaus *et al.* 2016.)

Method Development Reference:

Nielsen FK, Hansen CH, Fey JA, Hansen M, Jacobsen NW, Halling-Sørensen B, Björklund E, Styrishave B. (2012). "H295R cells as a model for steroidogenic disruption: a broader perspective using simultaneous chemical analysis of 7 key steroid hormones". Toxicology In Vitro 26:343-350.

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3.1. Data Interpretation

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Increased production of testosterone following interference with steroidogenesis as quantified by HPLC-MS/MS.

Analytical Elements:

CEETOX_H295R_TESTOSTERONE_up readout data was analyzed in the positive (gain of signal) fitting direction using DMSO controls as the baseline signal, and was reported as log2 fold-change increase in testosterone activity (compared between duplicate chemical treatment and plate-matched quadruplet DMSO controls). Data were $\log_2$-transformed, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest AIC value is considered the winning model and used in further analysis as the most appropriate predictor of assay effects. Testosterone stimulation was determined based on chemical-response data fulfilling the following criteria: the median of normalized response values at a single concentration exceeded the signal noise band (in this assay, any response over 6 times the baseline median absolute deviation in $\log_2$ fold-change; in the testosterone assay this created a 2.05 fold-change cut-off); if the modeled top of the curve (modl_tp) was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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CEETOX_H295R_CORTIC_up
CEETOX_H295R_CORTISOL_dn
CEETOX_H295R_CORTISOL_up
CEETOX_H295R_DHEA_dn
CEETOX_H295R_DHEA_up
CEETOX_H295R_DOC_dn
CEETOX_H295R_DOC_up

CEETOX_H295R_ESTRADIOL_up
CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_ESTRONE_up
CEETOX_H295R_MTT_Cytotoxicity_dn
CEETOX_H295R_MTT_Cytotoxicity_up
CEETOX_H295R_OHPREG_dn
CEETOX_H295R_OHPREG_up
CEETOX_H295R_OHPROG_dn
CEETOX_H295R_OHPROG_up
CEETOX_H295R_PREG_dn
CEETOX_H295R_PREG_up
CEETOX_H295R_PROG_dn
CEETOX_H295R_PROG_up
CEETOX_H295R_TESTO_dn
CEETOX_H295R_TESTO_up

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Assay Performance Measures:

One measure of assay performance is reproducibility. For the initial screening exercise that employed single-concentration and concentration-response phases, a Pearson's linear correlation test, using the median $\log_2$-fold change results from the MTC, was performed to demonstrate the concordance between the single-concentration and multi-concentration phases of screening. The correlation of maximum fold-change (r^2) is presented in Karmaus et al. 2016, Table 2. The correlation coefficients for each hormone between the two phases of that study, conducted independently, are presented by an average $r^2 = 0.70$. For testosterone, the $r^2 = 0.57$. These values demonstrate that the assay is highly reproducible.

Reference Chemicals / Predictive Capacity:

3.3. Assay Scope and Limitations

Chemical Library:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking [6].

4. Assay Documentation

4.1. References

[1] OECD (2011). Test No. 456 H295r Steroidogenesis Assay. Paris, OECD Publishing.

[2] Gracia, T., et al. (2006). Ecotoxicol Environ Saf 65(3): 293-305.
[3] Sanderson, J. T., et al. (2001). Toxicol Sci 61(1): 40-48. (PMID: 11294972)
[4] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[5] Mosmann, T. (1983). J Immunol Methods 65(1-2): 55-63.
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Type Culture Collection
DMSO, Dimethyl Sulfoxide
HPLC-MS/MS, High-Performance Liquid Chromatography, with tandem Mass Spectrometry
MTC, Maximum Tolerated Concentration
MTT, 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazoliumbromide
R^2, correlation coefficient, R-squared
SSMD, strictly standardized mean difference
Z', Robust Z-prime

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

17 January 2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

LTEA_HepaRG_THRSP_dn

Assay Title: Life Technologies / Expression Analysis HepaRG Cell-based High-Throughput Toxicogenomics Assay to Measure Thyroid Responsive Gene Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

A frequent concern with the extrapolation of in vitro assay data to in vivo toxicity is the potential lack of metabolic activity in the experimental platform used to assess chemical perturbations. Many xenobiotics impact biological systems following biotransformation in the liver, but most human hepatocyte cell lines express variable or negligible levels of liver-specific functions and P450 enzyme-related activities, making them unrepresentative models for in vivo toxicity. This assay was developed to further explore the extent to which liver specific functions and enzymes can impact toxicant responses in human hepatocytes. This assay assesses toxicogenomics in a metabolically competent human liver cell line (HepaRG) by screening the EPA ToxCast chemical library for gene-specific perturbations. Life Technologies exposed differentiated HepaRG cells in duplicate 96-well plates to 8-point half-log dilutions of test and control chemicals, and each assay includes positive controls for nuclear receptor activation (phenobarbital). Each plate assessed cell lysis (as measured by LDH release) following 48-hour incubation with a metabolically-activated cytotoxic agent (Aflatoxin B1). Following 48-hour concentration-response screening, cells were lysed and frozen and each plate was immediately shipped to Expression Analysis (Quintiles) labs for qRT-PCR analysis of changes in transcription levels for 93 genes related to biotransformation enzymes, nuclear receptors and NR mediated transporters, cell cycle regulation, and stress responses.

1.2. Assay Definition

Assay Throughput:

HepaRG cells are dispensed into 96-well microtiter plates and maintained in 770 media for 48 hours before media is removed and replaced with serum-free 750 induction media prior to test compound additions. Following 48-hour test exposures, 75 µL of spent media from each assay plate is transferred to black-walled microtiter plates to assess cytotoxicity (LDH release). Cells were then lysed with RLT buffer, sealed, frozen at -80 °C and sent for gene expression analysis (with universal human reference RNA added to each plate) using Fluidigm™ qRT-PCR.

Experimental System:

HepaRG is an immortalized cell line with a homogenous karyotype which was derived in 1999 from a hepatocarcinoma of a 66-year-old female Caucasian cholangiocarcinoma patient (Gripon et al. 2002). After proliferation, the cultures contain cells which retain the potential for inducible differentiation (with 2% DMSO addition) into hepatocytes and primitive biliary cells (Cerec et al. 2007, Gripon et al. 2002).

Xenobiotic Biotransformation Potential:

HepaRG cells have been shown to stably express various liver-specific functions after having reached confluency (Aninat et al. 2006). Under certain circumstances, HepaRG cells are capable of expressing cytochrome P450 enzymes (CYP1A2, 2B6, 2C9, 2D6, 2E1, and 3A4), metabolism-regulating nuclear receptors (AhR, PXR, CAR, and PPARα), phase 2 enzymes (UGT1A1, GSTA1, GSTA4, and GSTM1), and glutathione-related enzymes as well as liver-specific proteins (albumin, haptoglobin, and aldolase B), and functions (AFP and thioredoxin) have been detected (Aninat et al. 2006, Guillouzo et al. 2007). HepaRG cells seeded at high density show higher mRNA levels for these activities than in those seeded at low density and the highest values are observed in

differentiated cells exposed to 2% DMSO (Aninat et al. 2006, Guillouzo et al. 2007). With the exception of CYP2D6, the measured activities can be close or higher (CYP2B6 and CYP3A4) than those measured in primary human hepatocytes under similar culture conditions, and this capacity can be maintained in cultures over time (3-4 weeks) (Aninat et al. 2006, Anthérieu et al. 2010, Guillouzo et al. 2007, Josse et al. 2008, Lübberstedt et al. 2011). Therefore, HepaRG cells are thought to potentially possess both the metabolic performances of primary hepatocytes and growth capacity of hepatic cell lines (Andersson 2010, Guillouzo et al. 2007).

Basic Procedure:

Protocols

Cryopreserved HepaRG™ (LT catalog no. HPRGC10) in collagen (type I) coated 96-well plates were prepared according to standard methods and maintained in HepaRG™ 770 media for 48 hours post-plating. Prior to chemical additions, culture media was exchanged for serum-free 750 induction media and DMSO was back-added to stock chemicals to a final concentration of 0.5% for all dosing media. 8-point concentration-response curves were generated in half-log dose spacing ($10^{0.5}$-fold increments, maximum concentration 100 µM) for all chemicals. 48 hours after plating test cultures were dosed in duplicates and maintained in CO_2 incubator at 37 °C. Treated cells were imaged with an Essen IncuCyte™ FLR automated phase-contrast microscope with image analysis software at 24 and 48 hours post-exposure to assess morphological changes. The microscope was located inside the incubator to reduce cell disruption due to changes in environmental conditions. Each plate contained two total lysis control wells and four vehicle control wells (0.5% DMSO) as well as 1mM phenobarbital (induction positive control) and 100µM aflatoxin B1 (cytotoxicity positive control) in triplicate concentration response series. Total lysis controls were generated by removing spent culture media and replacing with 0.1% Triton X. Following 48-hour test exposures, 75µL of spent media from each assay plate was transferred to black-walled microtiter plates for cytotoxicity assessment using CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega) to assess LDH release. LDH activities were determined from each sample supernatant and were compared with mean responses of each total lysis control by calculation of total cell lysis activities for each replicate. Fluorescent emission was detected using a fluorometer set to optimal gain for each plate and raw data was converted from relative fluorescence units (RFU) to percent cytotoxicity, normalized to the vehicle and total lysis controls on each plate, as below:

% cytotoxicity = 100* [(Experimental Value - Culture Medium Background)/(Maximum LDH Release - Culture Medium Background)]

Cells were lysed with 75µL of RLT buffer, sealed, frozen at -80 °C and sent to EA for gene expression analysis using qRT-PCR using Fluidigm™ 96.96 microfluidic technology to determine ΔΔCt relative to DMSO and housekeeping genes. Each plate analyzed for gene expression included universal human reference RNA, a no template control and an enzyme-free control.

Proprietary Elements:

This assay is not proprietary. Cryopreserved HepaRG™ cells and media used are commercially available from ThermoFisher Scientific. Fluidigm 96.96 microfluidic technology is available from Fluidigm Corporation.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to activate endogenous thyroid signaling pathways, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to

provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Thermo Fisher Scientific (Formerly Life Technologies)
Global Headquarters
168 Third Avenue
Waltham, MA USA 02451

Phone:
781-622-1000
800-678-5599

Expression Analysis/Quintiles
US Headquarters
5827 South Miami Blvd
Morrisville, NC USA 27560

Phone:
919-998-7000

Assay Publication Year:

Assay Publication:

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The LTEA HepaRG thyroid responsive assay screened the ToxCast library of diverse environmental chemicals to probe for xenobiotic activity and potential to modulate expression of thyroid-hormone responsive gene (THRSP) in metabolically active human hepatocarcinoma cell line HepaRG. Bioactivity was monitored through analysis of cytotoxicity and morphological responses to 8-point half-log concentration series (100 to 0.1 µM) of duplicated chemical samples. Morphological changes were examined through phase-contrast microscopy at 24 and 48 hours post-exposure, and cytotoxicity was determined at 48 hours by LDH quantification. Metabolic activity of HepaRG cells was monitored by exposing the system to a 3-replicate 8-point concentration series of the mycotoxicant aflatoxin B1 (AFL), a cytotoxic agent requiring metabolic activation by cytochrome p450 dependent mixed-function oxidase to be converted to the reactive metabolite aflatoxin B1-8,9-epoxide (AFBO). A triplicate 8-point concentration series of the CAR-inducer phenobarbital was also included as a positive control for gene expression. Following the 48-hour concentration response screening, the cells were lysed and shipped to Expression Analysis/Quintiles for toxicogenomics analyses using real-time PCR conducted on Fluidigm 96.96 Dynamic Array IFCs (integrated fluidic circuits). This assay also included analysis of a chemical reference plate for gene expression inducers, including phenobarbital, omeprazole, fenofibric acid and chenodeoxycholic acid.

Scientific Principles:

Thyroid hormone signaling is essential for normal brain development both before and after birth and has profound effects on cellular metabolism in almost all organs. Thyroid hormone is important in lipid and glucose metabolism, lipolysis, and body weight, and variations in thyroid status in humans has been linked to significant changes in metabolic activity. Thyroidal regulation of metabolic pathways is a function of crosstalk with nuclear receptor signaling and thyroid hormone availability. One signaling pathway implicated in the link between thyroid status and body weight is regulated by THRSP, a primarily nuclear protein induced by TH and carbohydrate intake, which is closely related to lipid metabolism and breast cancer. An important component of an endocrine disruptor screening program should be the inclusion of assays designed to screen TH disrupting chemicals. The HepaRG cell line is a metabolically active hepatic system which was used to evaluate the potential for xenobiotic chemicals to alter THRSP activity. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous thyroid signaling by monitoring the expression of THRSP (fold-change relative to DMSO and housekeeping genes) following 48 hour incubation with test chemicals.

Method Development Reference:

Rotroff DM, Beam AL, Dix DJ, Farmer A, Freeman KM, Houck KA, Judson RS, LeCluyse EL, Martin MT, Reif DM. 2010. Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by ToxCast chemicals. Journal of Toxicology and Environmental Health, Part B 13:329-346. (PMID: 20574906)

Assay Quality Statistics:

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Inhibition of thyroid-hormone responsive gene (THRSP) monitored through qRT-PCR analysis of mRNA expression.

Analytical Elements:

Raw data were converted from raw relative fluorescence units (RFU) to percent cytotoxicity, normalized to the vehicle and total lysis controls on each plate, as in the following formula: % cytotoxicity = 100* [(Experimental - Culture Medium Background)/(Maximum LDH Release - Culture Medium Background)]. Gene expression was measured as ΔΔCt (log2 fold-change) relative to DMSO and housekeeping genes. Decreased (loss-of-signal) levels of mRNA indicate down regulation of THRSP by test chemical. Concentration-response relationships were determined based on a range of 8 chemical concentrations, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for

each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRa1_TRANS_up
ATG_THRb_TRANS2_up
LTEA_HepaRG_THRSP_up
NVS_NR_hTRa_Antagonist
Tox21_TR_LUC_GH3_Agonist
Tox21_TR_LUC_GH3_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [9].

4. Assay Documentation

4.1. References

[1] Gripon, P., et al. (2002). PNAS 99(24): 15655-15660. (PMID: 12432097)
[2] Cerec, V., et al. (2007). Hepatology 45(4): 957-967. (PMID: 17393521)
[3] Aninat, C., et al. (2006). Drug Metab Disposition 34(1): 75-83. (PMID: 16204462)
[4] Guillouzo, A., et al. (2007). Chem-Biol Interact 168(1): 66-73. (No PMID)
[5] Anthérieu, S., et al. (2010). Drug Metab Disposition 38(3): 516-525. (PMID: 20019244)
[6] Lübberstedt, M., et al. (2011). J Pharmacol Toxicol Methods 63(1): 59-68. (PMID: 19328226)
[7] Josse, R., et al. (2008). Drug Metab Disposition 36(6): 1111-1118. (PMID: 18347083)
[8] Andersson, T. B. (2010). Hepatocytes: Methods Protocols: 375-387. (PMID: 20645063)
[9] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EA, Expression Analysis
EDC, Endocrine disrupting chemicals
FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase
LDH, Lactate Dehydrogenase
LT, Life Technologies
NR, Nuclear Receptor
SULT, Sulfotransferases
THRSP, Thyroid-Hormone Responsive
UGT, UDP-glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

23 August 2016

Date of Revisions:

20 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

LTEA_HepaRG_THRSP_up

Assay Title: Life Technologies / Expression Analysis HepaRG Cell-based High-Throughput Toxicogenomics Assay to Measure Thyroid Responsive Gene Activation

1. Assay Descriptions

1.1. Overview

Assay Summary:

A frequent concern with the extrapolation of in vitro assay data to in vivo toxicity is the potential lack of metabolic activity in the experimental platform used to assess chemical perturbations. Many xenobiotics impact biological systems following biotransformation in the liver, but most human hepatocyte cell lines express variable or negligible levels of liver-specific functions and P450 enzyme-related activities, making them unrepresentative models for in vivo toxicity. This assay was developed to further explore the extent to which liver specific functions and enzymes can impact toxicant responses in human hepatocytes. This assay assesses toxicogenomics in a metabolically competent human liver cell line (HepaRG) by screening the EPA ToxCast chemical library for gene-specific perturbations. Life Technologies exposed differentiated HepaRG cells in duplicate 96-well plates to 8-point half-log dilutions of test and control chemicals, and each assay includes positive controls for nuclear receptor activation (phenobarbital). Each plate assessed cell lysis (as measured by LDH release) following 48-hour incubation with a metabolically-activated cytotoxic agent (Aflatoxin B1). Following 48-hour concentration-response screening, cells were lysed and frozen and each plate was immediately shipped to Expression Analysis (Quintiles) labs for qRT-PCR analysis of changes in transcription levels for 93 genes related to biotransformation enzymes, nuclear receptors and NR mediated transporters, cell cycle regulation, and stress responses.

1.2. Assay Definition

Assay Throughput:

HepaRG cells are dispensed into 96-well microtiter plates and maintained in 770 media for 48 hours before media is removed and replaced with serum-free 750 induction media prior to test compound additions. Following 48-hour test exposures, 75 µL of spent media from each assay plate is transferred to black-walled microtiter plates to assess cytotoxicity (LDH release). Cells were then lysed with RLT buffer, sealed, frozen at -80 °C and sent for gene expression analysis (with universal human reference RNA added to each plate) using Fluidigm™ qRT-PCR.

Experimental System:

HepaRG is an immortalized cell line with a homogenous karyotype which was derived in 1999 from a hepatocarcinoma of a 66-year-old female Caucasian cholangiocarcinoma patient [1]. After proliferation, the cultures contain cells which retain the potential for inducible differentiation (with 2% DMSO addition) into hepatocytes and primitive biliary cells [1, 2].

Xenobiotic Biotransformation Potential:

HepaRG cells have been shown to stably express various liver-specific functions after having reached confluency [3]. Under certain circumstances, HepaRG cells are capable of expressing cytochrome P450 enzymes (CYP1A2, 2B6, 2C9, 2D6, 2E1, and 3A4), metabolism-regulating nuclear receptors (AhR, PXR, CAR, and PPARα), phase 2 enzymes (UGT1A1, GSTA1, GSTA4, and GSTM1), and glutathione-related enzymes as well as liver-specific proteins (albumin, haptoglobin, and aldolase B), and functions (AFP and thioredoxin) have been detected [3, 4]. HepaRG cells seeded at high density show higher mRNA levels for these activities than in those seeded at low density and the highest values are observed in differentiated cells exposed to 2% DMSO [3, 4]. With the exception of CYP2D6, the measured activities can be close or higher (CYP2B6 and CYP3A4) than

those measured in primary human hepatocytes under similar culture conditions, and this capacity can be maintained in cultures over time (3-4 weeks) [3-7]. Therefore, HepaRG cells are thought to potentially possess both the metabolic performances of primary hepatocytes and growth capacity of hepatic cell lines [4, 8].

Basic Procedure:

Protocols

Cryopreserved HepaRG™ (LT catalog no. HPRGC10) in collagen (type I) coated 96-well plates were prepared according to standard methods and maintained in HepaRG™ 770 media for 48 hours post-plating. Prior to chemical additions, culture media was exchanged for serum-free 750 induction media and DMSO was back-added to stock chemicals to a final concentration of 0.5% for all dosing media. 8-point concentration-response curves were generated in half-log dose spacing ($10^{0.5}$-fold increments, maximum concentration 100 µM) for all chemicals. 48 hours after plating test cultures were dosed in duplicates and maintained in CO_2 incubator at 37 °C. Treated cells were imaged with an Essen IncuCyte™ FLR automated phase-contrast microscope with image analysis software at 24 and 48 hours post-exposure to assess morphological changes. The microscope was located inside the incubator to reduce cell disruption due to changes in environmental conditions. Each plate contained two total lysis control wells and four vehicle control wells (0.5% DMSO) as well as 1mM phenobarbital (induction positive control) and 100µM aflatoxin B1 (cytotoxicity positive control) in triplicate concentration response series. Total lysis controls were generated by removing spent culture media and replacing with 0.1% Triton X. Following 48-hour test exposures, 75µL of spent media from each assay plate was transferred to black-walled microtiter plates for cytotoxicity assessment using CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega) to assess LDH release. LDH activities were determined from each sample supernatant and were compared with mean responses of each total lysis control by calculation of total cell lysis activities for each replicate. Fluorescent emission was detected using a fluorometer set to optimal gain for each plate and raw data was converted from relative fluorescence units (RFU) to percent cytotoxicity, normalized to the vehicle and total lysis controls on each plate, as below:

% cytotoxicity = 100* [(Experimental Value - Culture Medium Background)/(Maximum LDH Release - Culture Medium Background)]

Cells were lysed with 75µL of RLT buffer, sealed, frozen at -80 °C and sent to EA for gene expression analysis using qRT-PCR using Fluidigm™ 96.96 microfluidic technology to determine ΔΔCt relative to DMSO and housekeeping genes. Each plate analyzed for gene expression included universal human reference RNA, a no template control and an enzyme-free control.

Proprietary Elements:

This assay is not proprietary. Cryopreserved HepaRG™ cells and media used are commercially available from ThermoFisher Scientific. Fluidigm 96.96 microfluidic technology is available from Fluidigm Corporation.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to activate endogenous thyroid signaling pathways, and is intended to provide information on a large number of diverse chemicals. Caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of chemical selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Thermo Fisher Scientific (Formerly Life Technologies)
Global Headquarters
168 Third Avenue
Waltham, MA USA 02451

Phone:
781-622-1000
800-678-5599

Expression Analysis/Quintiles
US Headquarters
5827 South Miami Blvd
Morrisville, NC USA 27560

Phone:
919-998-7000

Assay Publication Year:

Assay Publication:

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The LTEA HepaRG thyroid responsive assay screened the ToxCast library of diverse environmental chemicals to probe for xenobiotic activity and potential to modulate expression of thyroid-hormone responsive gene (THRSP) in metabolically active human hepatocarcinoma cell line HepaRG. Bioactivity was monitored through analysis of cytotoxicity and morphological responses to 8-point half-log concentration series (100 to 0.1 µM) of duplicated chemical samples. Morphological changes were examined through phase-contrast microscopy at 24 and 48 hours post-exposure, and cytotoxicity was determined at 48 hours by LDH quantification. Metabolic activity of HepaRG cells was monitored by exposing the system to a 3-replicate 8-point concentration series of the mycotoxicant aflatoxin B1 (AFL), a cytotoxic agent requiring metabolic activation by cytochrome p450 dependent mixed-function oxidase to be converted to the reactive metabolite aflatoxin B1-8,9-epoxide (AFBO). A triplicate 8-point concentration series of the CAR-inducer phenobarbital was also included as a positive control for gene expression. Following the 48 hour concentration response screening, the cells were lysed and shipped to Expression Analysis/Quintiles for toxicogenomics analyses using real-time PCR conducted on Fluidigm 96.96 Dynamic Array IFCs (integrated fluidic circuits). This assay also included analysis of a chemical reference plate for gene expression inducers, including phenobarbital, omeprazole, fenofibric acid and chenodeoxycholic acid.

Scientific Principles:

Thyroid hormone signaling is essential for normal brain development both before and after birth and has profound effects on cellular metabolism in almost all organs. Thyroid hormone is important in lipid and glucose metabolism, lipolysis, and body weight, and variations in thyroid status in humans has been linked to significant changes in metabolic activity. Thyroidal regulation of

metabolic pathways is a function of crosstalk with nuclear receptor signaling and thyroid hormone availability. One signaling pathway implicated in the link between thyroid status and body weight is regulated by THRSP, a primarily nuclear protein induced by TH and carbohydrate intake, which is closely related to lipid metabolism and breast cancer. An important component of an endocrine disruptor screening program should be the inclusion of assays designed to screen TH disrupting chemicals. The HepaRG cell line is a metabolically active hepatic system which was used to evaluate the potential for xenobiotic chemicals to alter THRSP activity. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous thyroid signaling by monitoring the expression of THRSP (fold-change relative to DMSO and housekeeping genes) following 48-hour incubation with test chemicals.

Method Development Reference:

Rotroff DM, Beam AL, Dix DJ, Farmer A, Freeman KM, Houck KA, Judson RS, LeCluyse EL, Martin MT, Reif DM. 2010. Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by ToxCast chemicals. Journal of Toxicology and Environmental Health, Part B 13:329-346.

Assay Quality Statistics:

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Activation of thyroid-hormone responsive gene (THRSP) monitored through qRT-PCR analysis of mRNA expression.

Analytical Elements:

Raw data were converted from raw relative fluorescence units (RFU) to percent cytotoxicity, normalized to the vehicle and total lysis controls on each plate, as in the following formula: % cytotoxicity = 100* [(Experimental - Culture Medium Background)/(Maximum LDH Release - Culture Medium Background)]. Gene expression was measured as ΔΔCt (log2 fold-induction) relative to DMSO and housekeeping genes. Increased (gain-of-signal) levels of mRNA indicate upregulation of THRSP by test chemical. Concentration-response relationships were determined based on a range of 8 chemical concentrations, and all statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRa1_TRANS_up

ATG_THRb_TRANS2_up
LTEA_HepaRG_THRSP_dn
LTEA_HepaRG_THRSP_up
NVS_NR_hTRa_Antagonist
Tox21_TR_LUC_GH3_Agonist
Tox21_TR_LUC_GH3_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. [9].

4. Assay Documentation

4.1. References

[1] Gripon, P., et al. (2002). PNAS 99(24): 15655-15660. (PMID: 12432097)
[2] Cerec, V., et al. (2007). Hepatology 45(4): 957-967. (PMID: 17393521)
[3] Aninat, C., et al. (2006). Drug Metab Disposition 34(1): 75-83. (PMID: 16204462)
[4] Guillouzo, A., et al. (2007). Chem-Biol Interact 168(1): 66-73. (No PMID)
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[6] Lübberstedt, M., et al. (2011). J Pharmacol Toxicol Methods 63(1): 59-68. (PMID: 19328226)
[7] Josse, R., et al. (2008). Drug Metab Disposition 36(6): 1111-1118. (PMID: 18347083)
[8] Andersson, T. B. (2010). Hepatocytes: Methods Protocols: 375-387. (PMID: 20645063)
[9] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATCC, American Tissue Culture Collection
CYP, Cytochrome P450s
DMSO, Dimethyl Sulfoxide
EA, Expression Analysis
EDC, Endocrine disrupting chemicals
FBS, Fetal Bovine Serum
GST, Glutathione S-Transferase
LDH, Lactate Dehydrogenase
LT, Life Technologies

NR, Nuclear Receptor
SULT, Sulfotransferases
THRSP, Thyroid-Hormone Responsive
UGT, UDP-glucuronosyltransferase

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

23 August 2016

Date of Revisions:

20 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

NVS_NR_bER

Assay Title: NovaScreen Bovine Estrogen Receptor HTS Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for xenoestrogenic activity. This assay format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a radiolabeled ligand from the ligand-binding domain of the estrogen receptor. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 18 hours in a 96-well plate.

1.2. Assay Definition

Assay Throughput:

This is a biochemical (cell-free) format, using 96-well plates to incubate radiolabeled ligand with estrogen receptor alpha for 18 hours to measure displacement of estradiol by test chemicals in a competitive binding assay.

Experimental System:

ERα nuclear protein, derived from bovine uterine membranes

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: Bovine uterine membranes
Radioligand: [3H] Estradiol
Final ligand concentration - [0.7 nM]
Non-specific Determinant: 17β-Estradiol - [10 nM]
Positive Control: 17β-Estradiol

Methods:

Incubation Conditions: Reactions are carried out in 10 mM TRIS-HCl (pH 7.4 containing 1.5 mM EDTA, 1.0 mM DTT, and 25 mM sodium molybdate at 0-4 °C for 18 hours. The reaction is terminated by the addition of dextran-coated charcoal and incubated for 20 minutes at 0-4 °C. The reaction mixtures are centrifuged and the radioactivity bound in the supernatant is assessed and compared to control values in order to ascertain any interactions of test compound with the estradiol binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective cell membrane and biotransformation capacity expected in in vivo or cell-based systems. The potential for a particular compound to affect changes in estrogen

signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen nuclear receptor bovine estrogen receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (96-well microplate) format to screen the ToxCast chemical library for xenoestrogenic interaction with bovine nuclear receptors. An initial screening run was conducted exposing estrogen receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using radioligand detection (via liquid scintillation counting) of displacement of [^{3}H]-Estradiol. 17β-Estradiol (E2) was used as a positive control. If the response signal differed by over 30% or varied by a minimum of 3.0 baseline median absolute deviations (3BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding activity with estrogen nuclear receptor alpha (ERα) derived from bovine uterine membranes.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenoestrogenic ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor activity in early life is a molecular initiating event (MIE) leading to breast cancer in both animal and human models and to endometrial carcinoma in the mouse, and ER agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Haji, M., Kato, K., Nawata, H., & Ibayashi, H. (1981). "Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat". Brain Res 204(2), 373-386. (PMID: 6780133)

O'Keefe, J. A., & Handa, R. J. (1990). "Transient elevation of estrogen receptors in the neonatal rat hippocampus". Dev Brain Res 57(1), 119-127. (PMID: 2090365)

Assay Quality Statistics:

Neutral control well median response value, by plate:	2637.21
Neutral control median absolute deviation, by plate:	74.86
Positive control well median response value, by plate:	521.88
Positive control well median absolute deviation, by plate:	21.18
Z' (median across all plates, using positive control wells):	0.86
SSMD (median across all plates, using positive control wells):	-26
Signal-to-noise (median across all plates, using positive control wells):	-29.49
Signal-to-background (median across all plates, using positive control wells):	0.19
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive radioligand binding of [3H] Estradiol (positive control) with estrogen receptor α obtained from bovine uterine membranes and measured by radiometric detection.

Analytical Elements:

The NVS_NR_bER assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of [^{3}H] 17β-estradiol binding. Raw data values were normalized to DMSO (neutral control) and reported as percent of 17-β Estradiol (positive control) binding capacity. Following initial screening of test compounds at single concentration (25 µM), if the chemical response was >30% of the solvent control (DMSO) activity or at least 2 baseline median average deviations (2BMAD), the chemical was considered active against the estrogen receptor and was tested in a concentration-response assay for ER binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is

considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 8
Target (nominal) number of replicates: 1
Standard minimum concentration tested: 0.02 µM
Standard maximum concentration tested: 50 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.4.03
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 24.17

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes

57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	No
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	No
98-54-4	4-tert-Butylphenol	NA	Active	No
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	No
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	Yes
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	No
94-26-8	Butylparaben	NA	Active	No
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	No
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	Yes
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	No

50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	No
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	No
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	No
72-33-3	Mestranol	NA	Active	No
72-43-5	Methoxychlor	Very Weak	Active	No
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	No
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	Yes
57-30-7	Phenobarbital sodium	Inactive	NA	Yes
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	Yes
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	12	22
Inactive	12	6

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	22	15
Inactive	2	13

In Vitro Sensitivity = 35.2%
In Vitro Specificity = 33.3%
Balanced Accuracy = 34.3%

In Vivo Sensitivity = 59.5%
In Vivo Specificity = 86.7%
Balanced Accuracy = 73.1%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to

environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
E2, Estradiol
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 710

NVS_NR_cAR

Assay Title: NovaScreen Chimpanzee Androgen Receptor Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for xenobiotic activity. The NovaScreen NR assays utilized a cell-free platform to screen a diverse chemical library for nuclear receptor activity in a high-throughput (96-well plate) format. This format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a radiolabeled ligand from the ligand-binding domain of the androgen receptor following 72-hour incubation with test chemicals. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity.

1.2. Assay Definition

Assay Throughput:

Recombinant chimpanzee androgen receptor incubated in 96-well microtiter plates for 72 hours prior to measuring displacement of R1881 radiolabeled ligand by test compounds

Experimental System:

Baculovirus (Sf9/Sf21 cell line) derived recombinant chimpanzee AR

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: Recombinant chimpanzee androgen receptor

Radioligand: [^{3}H]Methyltrienolone (R1881)

Non-specific Determinant: Methyltrienolone (R1881)

Reference Compound: Methyltrienolone (R1881)

Positive Control: Methyltrienolone (R1881)

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity expected in in vivo systems. The potential for a particular compound to affect changes in androgen signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office

940 Winter St.

Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Hartig, P., Cardon, M., Blystone, C., Gray, L., & Wilson, V. (2008). High throughput adjustable 96-well plate assay for androgen receptor binding: a practical approach for EDC screening using the chimpanzee AR. Toxicology letters, 181(2), 126-131.

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen nuclear receptor chimpanzee androgen receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (96-well microplate) format to screen the ToxCast chemical library for xenobiotic androgen receptor interaction with chimpanzee-derived nuclear receptors. An initial screening run was conducted exposing androgen receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using radioligand detection (via liquid scintillation counting) of displacement of [^{3}H]-Methyltrienolone (R1881). R1881 was used as a positive control. If the response signal differed by over 30% or varied by a minimum of 3.0 baseline median absolute deviations (3BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding activity with recombinant chimpanzee androgen receptor.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting androgen signaling pathways. The androgen receptor mediates gene expression in response to androgen exposures, and modulates the activity for a wide variety of physiological processes, particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility.

The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenobiotic androgen receptor ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Hartig, P., Cardon, M., Lambright, C., Bobseine, K., Gray, L., & Wilson, V. (2007). Substitution of synthetic chimpanzee androgen receptor for human androgen receptor in competitive binding and transcriptional activation assays for EDC screening. Toxicology letters, 174(1), 89-97.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5105.21
Neutral control median absolute deviation, by plate:	89.69
Positive control well median response value, by plate:	284.18
Positive control well median absolute deviation, by plate:	21.96
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.89
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-39.65
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive radioligand binding of [^{3}H] Methyltrienolone (R1881) (positive control) with recombinant chimpanzee androgen receptor.

Analytical Elements:

The NVS_NR_cAR assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of [^{3}H] Methyltrienolone (R1881) binding. Raw data values were normalized to DMSO (neutral control) and reported as percent of R1881 (positive control; calculated per plate, or as a median of positive control wells from all plates combined)

binding capacity. Following initial screening of test compounds at single concentration (25 µM), if the chemical response was >30% of the solvent control (DMSO) activity or at least 3 baseline median average deviations (3BMAD), the chemical was considered active against the androgen receptor and was tested in a concentration-response assay for AR binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.68
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [2].

4. Assay Documentation

4.1. References

[1] Veldscholte, J., et al. (1992). J Steroid Biochem Mol Biol 41: 665-669. (PMID: 1562539)
[2] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 711

NVS_NR_hAR

Assay Title: NovaScreen Human Androgen Receptor Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for xenobiotic activity. The NovaScreen NR assays utilized a cell-free platform to screen a diverse chemical library for nuclear receptor activity in a high-throughput (96-well plate) format. This format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a radiolabeled ligand from the ligand-binding domain of the androgen receptor following 20-hour incubation with test chemicals. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity.

1.2. Assay Definition

Assay Throughput:

Human androgen receptor incubated in 96-well microtiter plates for 20 hours prior to measuring displacement of R1881 radiolabeled ligand by test compounds.

Experimental System:

AR nuclear protein, derived from human prostate adenocarcinoma (LNCaP cells) cell line (see Caveats for further discussion on this cell line).

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: LNCaP cells
Radioligand: [^{3}H]Methyltrienolone (R1881) (70-87 Ci/mmol)
Final Ligand Concentration – [0.5 nM]
Non-specific Determinant: Methyltrienolone (R1881) - [200 nM]
Reference Compound: Methyltrienolone (R1881)
Positive Control: Methyltrienolone (R1881)

Methods:

Incubation Conditions: Reactions are carried out in 25 mM HEPES buffer (pH 7.4) containing 1.0 mM EDTA, 10 mM sodium molybdate, 10% glycerol, and 0.5 mM PMSF at 0-4°C for 18-20 hours. The reaction is terminated by the addition of dextran-coated charcoal and incubated for 7 minutes at 0-4°C. The reaction mixtures are centrifuged and the radioactivity bound in the supernatant is compared to control values in order to ascertain any interactions of test compound with the testosterone binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity expected in in vivo systems. The potential for a particular compound to affect changes in androgen signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

LNCaP cells have been shown to contain a single point mutation changing the sense of codon 868 (Thr to Ala) in the ligand binding domain. The androgen receptor in these cells is known to bind to progestagens and estrogens despite the absence of estrogen or progestagen receptors, and binding to androgens, progestagens, estrogens and several antiandrogens to the mutated androgen receptor protein activates the expression of an androgen-regulated reporter gene (GRE-tk-CAT), indicating that the mutation directly affects both binding specificity and the induction of gene expression. Consequently, the androgen receptor in these cells is known to bind steroid hormones other than androgens (Veldscholte et al. 1992).

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None Reported

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen nuclear receptor human androgen receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (96-well microplate) format to screen the ToxCast chemical library for xenobiotic androgen receptor interaction with human-derived nuclear receptors. An initial screening run was conducted exposing androgen receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using radioligand detection (via liquid scintillation counting) of displacement of [^{3}H]-Methyltrienolone (R1881). R1881 was used as a positive control. If the response signal differed by over 30% or varied by a minimum of 3.0 baseline median absolute deviations (3BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top

concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding activity with androgen nuclear receptor (AR) derived from a human prostate adenocarcinoma cell line (LNCaP).

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting androgen signaling pathways. The androgen receptor mediates gene expression in response to androgen exposures, and modulates the activity for a wide variety of physiological processes, particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility.

The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenobiotic androgen receptor ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Traish, A. M., Enzio Muller, R., & Wotiz, H. H. (1986). "Binding of 7 α, 17 α-Dimethyl-19-Nortestosterone (Mibolerone) to Androgen and Progesterone Receptors in Human and Animal Tissues". Endocrinology 118(4), 1327-1333.

Zava, D., Landrum, B., Horwitz, K., & McGuire, W. (1979). "Androgen Receptor Assay with [3H] Methyltrienolone (R1881) in the Presence of Progesterone Receptors". Endocrinology 104(4), 1007-1012.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3618.46
Neutral control median absolute deviation, by plate:	163.175
Positive control well median response value, by plate:	197.325
Positive control well median absolute deviation, by plate:	21.690
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.85

Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-23.16
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive radioligand binding of [^{3}H] Methyltrienolone (R1881) (positive control) with androgen receptor obtained from human prostate adenocarcinoma cell line LNCaP

Analytical Elements:

The NVS_NR_hAR assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of [^{3}H] Methyltrienolone (R1881) binding. Raw data values were normalized to DMSO (neutral control) and reported as percent of R1881 (positive control; calculated per plate, or as a median of positive control wells from all plates combined) binding capacity. Following initial screening of test compounds at single concentration (25 µM), if the chemical response was >30% of the solvent control (DMSO) activity or at least 3 baseline median average deviations (3BMAD), the chemical was considered active against the androgen receptor and was tested in a concentration-response assay for AR binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480

OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.65
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.90

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [2].

4. Assay Documentation

4.1. References

[1] Veldscholte, J., et al. (1992). J Steroid Biochem Mol Biol 41: 665-669. (PMID: 1562539)
[2] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation;

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA

6. Supporting Information (existing annotations):

Assay Endpoint ID: 714

NVS_NR_hER

Assay Title: NovaScreen Human Estrogen Receptor HTS Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for xenoestrogenic activity. This assay format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a radiolabeled ligand from the ligand-binding domain of the estrogen receptor. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 18 hours in a 96-well plate.

1.2. Assay Definition

Assay Throughput:

Human ERα nuclear protein incubated in 96-well microtiter plates for 18 hours prior to measuring displacement of radiolabeled 17β-Estradiol by test compounds.

Experimental System:

ERα nuclear protein, derived from human breast adenocarcinoma (MCF-7) cell line.

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: MCF-7 cells
Radioligand: [3H] Estradiol
Final Ligand Concentration – [0.1 nM]
Non-specific Determinant: 17β-Estradiol - [300 nM]
Reference Compound: 17β-Estradiol
Positive Control: 17β-Estradiol

Methods:

Incubation Conditions: Reactions are carried out in 10 mM TRIS-HCl (pH 7.4) containing 1.5 mM EDTA, 1.0 mM DTT, and 25 mM sodium molybdate at 0-4 °C for 18 hours. The reaction is terminated by the addition of dextran-coated charcoal and incubated for 20 minutes at 0-4°C. The reaction mixtures are centrifuged and the radioactivity bound in the supernatant is assessed and compared to control values in order to ascertain any interactions of test compound with the estradiol binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective cell membrane and biotransformation capacity expected in in vivo and cell-based systems. The potential for a particular compound to affect changes in

estrogen signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen nuclear receptor human estrogen receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (96-well microplate) format to screen the ToxCast chemical library for xenoestrogenic interaction with estrogen receptors. An initial screening run was conducted exposing human estrogen receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using radioligand detection (via liquid scintillation counting) of displacement of [^{3}H]-Estradiol. 17β-Estradiol (E2) was used as a positive control. If the response signal differed by over 30% or varied by a minimum of 3.0 baseline median absolute deviations (3BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding activity with estrogen nuclear receptor alpha (ERα) derived from MCF-7 human breast adenocarcinoma lysate.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput

format. This assay is designed to help identify environmental compounds with a capacity for xenoestrogenic ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor activity in early life is a molecular initiating event (MIE) leading to breast cancer in both animal and human models and to endometrial carcinoma in the mouse, and ER agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Haji, M., Kato, K., Nawata, H., & Ibayashi, H. (1981). "Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat". Brain Res 204(2), 373-386. (PMID: 6780133)

O'Keefe, J. A., & Handa, R. J. (1990). "Transient elevation of estrogen receptors in the neonatal rat hippocampus". Dev Brain Res 57(1), 119-127. (PMID: 2090365)

Assay Quality Statistics:

Neutral control well median response value, by plate:	4467.25
Neutral control median absolute deviation, by plate:	93.76
Positive control well median response value, by plate:	293.52
Positive control well median absolute deviation, by plate:	20.89
Z' (median across all plates, using positive control wells):	0.9
SSMD (median across all plates, using positive control wells):	-36
Signal-to-noise (median across all plates, using positive control wells):	-40.33
Signal-to-background (median across all plates, using positive control wells):	0.08
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive displacement of [3H] Estradiol (positive control) with estrogen receptor α obtained from human breast adenocarcinoma cell line (MCF-7) as measured by detection of radioligand.

Analytical Elements:

The NVS_NR_hER assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of [^{3}H] 17β-estradiol binding. Raw data values were normalized as percent of 17-β Estradiol (positive control) binding capacity. If the chemical interaction was >30% of the solvent control (DMSO) or if the signal varied by more than 3.0 median average deviations (3MAD), the chemical was considered active against the estrogen receptor and was tested in a concentration-response assay for ER binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model

which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 8
Target (nominal) number of replicates: 1
Standard minimum concentration tested: 0.02 µM
Standard maximum concentration tested: 50 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 4.07
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 24.43

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes

57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	Yes
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	No
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	No
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	No
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	Yes
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	No
77-40-7	Bisphenol B	Weak	Active	No
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	No
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	No
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	No
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes

50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	Yes
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	18	17
Inactive	8	10

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	22	15
Inactive	4	12

In Vitro Sensitivity = 51.4%
In Vitro Specificity = 55.6%
Balanced Accuracy = 53.5%

In Vivo Sensitivity = 59.5%
In Vivo Specificity = 75%
Balanced Accuracy = 67.3%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to

environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
E2, Estradiol
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 715

NVS_NR_hFXR_Agonist

Assay Title: NovaScreen Human Farnesoid x Receptor Alpha (FXR) Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for bioactivity. The NVS NR human farnesoid x receptor (FXR, NR1H4) agonist assay format allows for an efficient screening of thousands of chemicals for the ability to competitively bind to the ligand-binding domain of a xenobiotic sensing nuclear receptor. This assay was developed to screen the ToxCast chemical library for potential farnesoid x receptor ligand-binding activity using a TR-FRET competitive displacement assay and a known FXR receptor agonist (Chenodeoxycholic Acid, CDCA) as a reference compound. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 1 hour in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

Human FXR ligand-binding domain (LBD) incubated in 384-well microtiter plates for 1 hour prior to measuring ligand dependent binding of cofactor to the receptor using TR-FRET.

Experimental System:

GST tagged Human- Farnesoid X Receptor Ligand Binding Domain

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials :

Receptor Source: Human- Farnesoid X Receptor Ligand Binding Domain, GST tagged
Cofactor: Fluorescein-SRC2
Reference Agonist: Chenodeoxycholic Acid (CDCA)
Reaction: Agonist dependent binding of receptor to cofactor
Method: Time-Resolved Fluorescence Resonance Energy Transfer

Methods:

Incubation Conditions: Reactions containing receptor, fluorescein-labeled coactivator peptide, terbium labeled antibody and test or control compounds are carried out in buffer (pH 7.5) for 60 minutes at room temperature. Agonist binding causes receptor to bind with coactivator. The gain of TRF between coactivator peptide and terbium-labeled anti-GST antibody is assessed and compared to control values in order to ascertain any interactions of test compound with the ligand binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity expected in in vivo systems. The potential for a particular compound to affect changes in FXR signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen hFXR ligand-binding agonist assay used a biochemical (cell-free) platform in high-throughput (384-well microplate) format to screen the ToxCast chemical library for xenobiotic interaction with human-derived FXR nuclear receptors. An initial screening run was conducted exposing receptors to 25 µM of each chemical (in duplicate). FXR ligand binding by test chemicals was analyzed using fluorescence resonance energy transfer to measure ligand-dependent cofactor recruitment by test compounds. Reactions involved GST-tagged ligand-binding domain of human FXR, fluorescein-labeled coactivator and terbium-labeled GST antibody. Activity was reported as percent of CDCA (a known FXR agonist) ligand-binding and coactivator recruitment. If the response signal differed by over 30% or varied by a minimum of 3.0 baseline median absolute deviations (3BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding of nuclear receptor FXR.

Scientific Principles:

Farnesoid-X-receptor (FXR) is a ligand-activated nuclear receptor which regulates the expression of genes involved in bile acid homeostasis and has a role in the regulation of glucose and lipid metabolic pathways. FXR is primarily expressed in the liver, kidney, intestine and adrenal cortex, and regulates the expression of target genes by binding either as a monomer or as a heterodimer

with the retinoid X receptor (RXR). Numerous studies have reported that FXR exerts protective function during cholestasis, diabetes, liver regeneration, and cancer. The FXR-RXR heterodimer, when bound to DNA, can act as transcriptional activators or inhibitors. FXR is activated by bile acids and the main endogenous ligand for FXR is chenodeoxycholic acid (CDCA). FXR reduces bile acid concentration in the liver by repressing genes involved in bile acid synthesis and regulates lipid metabolism. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenobiotic FXR ligand-binding activity.

Method Development Reference:

Urizar, N. L., A. B. Liverman, T. D. D'Nette, F. V. Silva, P. Ordentlich, Y. Yan, F. J. Gonzalez, R. A. Heyman, D. J. Mangelsdorf and D. D. Moore (2002). "A natural product that lowers cholesterol as an antagonist ligand for FXR." Science 296(5573): 1703-1706.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.875
Neutral control median absolute deviation, by plate:	0.0178
Positive control well median response value, by plate:	1.761
Positive control well median absolute deviation, by plate:	0.0682
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.79
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	54.3
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	2.22
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Time-resolved Fluorescent Energy Resonance Transfer between terbium-labeled antibody, a fluorescein-labeled coactivator peptide, and human FXR ligand-binding domain

Analytical Elements:

The NVS_NR_hFXR assay results were analyzed as loss-of-signal in TR_FRET assays where the endpoint measured was inhibition of CDCA binding. Raw data values were normalized as percent of CDCA (positive control, 100% binding activity). If the chemical interaction was >30% of the solvent control (DMSO) activity or at least 3*baseline median average deviations (3BMAD), the chemical was considered active against the receptor and was tested in a concentration-response assay for FXR binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC)

value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up
ATG_FXR_TRANS_up
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_0480
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50.0 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	13.88
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	83.29

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical

inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
CDCA, Chenodeoxycholic Acid
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
GST, Glutathione S-Transferase
FXR, Farnesoid X Receptor
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 October 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

NVS_NR_hFXR_Antagonist

Assay Title: NovaScreen Human Farnesoid x Receptor Alpha (FXR) Ligand-Binding Antagonist Screening Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for bioactivity. The NVS NR human farnesoid x receptor (FXR, NR1H4) agonist assay format allows for an efficient screening of thousands of chemicals for the ability to competitively bind to the ligand-binding domain of a xenobiotic sensing nuclear receptor. This assay was developed to screen the ToxCast chemical library for potential farnesoid x receptor ligand-binding activity using a TR-FRET competitive displacement assay and a known FXR receptor agonist (Chenodeoxycholic Acid, CDCA) as a reference compound. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 1 hour in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

Human FXR ligand-binding domain (LBD) incubated in 384-well microtiter plates for 1 hour prior to measuring ligand dependent binding of cofactor to the receptor using TR-FRET.

Experimental System:

GST tagged Human- Farnesoid X Receptor Ligand Binding Domain

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials :

Receptor Source: Human-GST tagged Farnesoid X Receptor Ligand Binding Domain
Cofactor: Fluorescein-SRC2
Receptor Source: Human- Farnesoid X Receptor Ligand Binding Domain
Ligand and concentration: Chenodeoxycholic Acid (CDCA), 12.5 µM
Reaction: Ligand dependent binding of cofactor to the receptor-ligand complex.
Method: Time-Resolved Fluorescence Resonance Energy Transfer

Methods:

Incubation Conditions: Reactions containing receptor, agonist, fluorescein-labeled coactivator peptide, terbium labeled antibody and test or control compounds are carried out in buffer (pH 7.5). The reaction is started by addition of agonist and incubated for 60 minutes at room temperature. Agonist binding causes receptor to bind to coactivator. The loss of FRET between coactivator peptide and terbium-labeled antibody is assessed and compared to control values in order to ascertain any interactions of test compound with the ligand binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity expected in in vivo systems. The potential for a particular compound to affect changes in FXR signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen hFXR ligand-binding antagonist assay used a biochemical (cell-free) platform in high-throughput (384-well microplate) format to screen the ToxCast chemical library for xenobiotic interaction with human-derived FXR nuclear receptors. An initial screening run was conducted exposing receptors to 25 µM of each chemical (in duplicate). FXR ligand binding by test chemicals was analyzed using fluorescence resonance energy transfer to measure loss-of-signal competitive displacement of agonist (CDCA) by test compounds. Reactions involved GST-tagged ligand-binding domain of human FXR, fluorescein-labeled coactivator and terbium-labeled GST antibody. Activity was reported as percent of Guggulsterone (a known FXR antagonist) binding. If the response signal differed by over 30% or varied by a minimum of 3.0 baseline median absolute deviations (3BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding of nuclear receptor FXR.

Scientific Principles:

Farnesoid-X-receptor (FXR) is a ligand-activated nuclear receptor which regulates the expression of genes involved in bile acid homeostasis and has a role in the regulation of glucose and lipid metabolic pathways. FXR is primarily expressed in the liver, kidney, intestine and adrenal cortex,

and regulates the expression of target genes by binding either as a monomer or as a heterodimer with the retinoid X receptor (RXR). Numerous studies have reported that FXR exerts protective function during cholestasis, diabetes, liver regeneration, and cancer. The FXR-RXR heterodimer, when bound to DNA, can act as transcriptional activators or inhibitors. FXR is activated by bile acids and the main endogenous ligand for FXR is chenodeoxycholic acid (CDCA). FXR reduces bile acid concentration in the liver by repressing genes involved in bile acid synthesis and regulates lipid metabolism. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenobiotic FXR ligand-binding activity.

Method Development Reference:

Urizar, N. L., A. B. Liverman, T. D. D'Nette, F. V. Silva, P. Ordentlich, Y. Yan, F. J. Gonzalez, R. A. Heyman, D. J. Mangelsdorf and D. D. Moore (2002). "A natural product that lowers cholesterol as an antagonist ligand for FXR." Science 296(5573): 1703-1706.

Assay Quality Statistics:

Neutral control well median response value, by plate:	194004
Neutral control median absolute deviation, by plate:	348.411
Positive control well median response value, by plate:	2908.25
Positive control well median absolute deviation, by plate:	333.956
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.93
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-110.84
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Time-resolved Fluorescent Energy Resonance Transfer reactions containing ligand-binding domain of human FXR, agonist, fluorescein-labeled coactivator peptide, terbium labeled antibody and test or control compounds.

Analytical Elements:

The NVS_NR_hFXR assay results were analyzed as loss-of-signal in TR_FRET assays where the endpoint measured was inhibition of CDCA binding. Raw data values were normalized as percent of Guggulsterone (positive control, 100% binding activity). If the chemical interaction was >30% of the solvent control (DMSO) activity or at least 3*baseline median average deviations (3BMAD), the chemical was considered active against the receptor and was tested in a concentration-response assay for FXR binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-

loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up
ATG_FXR_TRANS_up
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_0480
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.213
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.28

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the

latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
CDCA, Chenodeoxycholic Acid
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
GST, Glutathione S-Transferase
FXR, Farnesoid X Receptor
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

29 August 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 718

NVS_NR_hPPARa

Assay Title: NovaScreen Human Peroxisome Proliferator-activated Receptor Alpha (PPARα) Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for bioactivity. The NVS NR human PPARα assay format allows for an efficient screening of thousands of chemicals for the ability to competitively bind to the ligand-binding domain of a xenobiotic sensing nuclear receptor. This assay was developed to screen the ToxCast chemical library for potential to interfere with peroxisome proliferator-activated receptor alpha (PPARα) ligand-binding activity using a TR-FRET competitive displacement assay and a known PPARα receptor agonist (GW7647) as a reference compound. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 1 hour in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

Human PPARα ligand-binding domain (LBD) incubated in 384-well microtiter plates for 1 hour prior to measuring ligand dependent binding of cofactor to the receptor using TR-FRET.

Experimental System:

Ligand-binding domain of human PPARα

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:
Receptor Source: Human PPARα ligand-binding domain (LBD)
Ligand: Fluormone PPAR green, 20nM
Reference Compound: GW7647
Reaction: Ligand dependent binding of cofactor to the receptor.
Method: Time-Resolved Fluorescence Resonance Energy Transfer

Methods:
Incubation Conditions: Reactions are carried out in buffer (pH 7.5) for 60 minutes at room temperature. The loss of FRET between fluoromone and Tb-labeled anti-PPAR antibody is assessed and compared to control values in order to ascertain any interactions of test compound with the ligand binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity

expected in in vivo systems. The potential for a particular compound to affect changes in PPAR signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen PPARα ligand-binding assay used a biochemical (cell-free) platform in high-throughput (384-well microplate) format to screen the ToxCast chemical library for xenobiotic interaction with human-derived nuclear receptors. An initial screening run was conducted exposing receptors to 25 µM of each chemical (in duplicate). PPARα ligand binding by test chemicals was analyzed using fluorescence resonance energy transfer to measure displacement of a fluorescent small molecule pan-PPAR ligand (Fluormone™ PPAR Green) and human PPARα ligand-binding domain by test compounds. Activity was reported as percent of GW7647 (a potent PPARα agonist) PPARα ligand-binding. If the response signal differed by over 30% or varied by a minimum of 2.0 baseline median absolute deviations (2BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding of nuclear receptor PPARα.

Scientific Principles:

Peroxisome proliferator-activated receptor alpha (PPARα) is a ligand-activated nuclear receptor which regulates the expression of genes involved in fatty acid-oxidation and is a major regulator of energy homeostasis. PPARα plays a crucial role in the regulation of proteins involved in fatty acid transport and hepatic uptake, is expressed predominantly in metabolically active tissues, including liver, kidney, skeletal muscle, and brown fat and is a target for hyperlipidemia drugs, fatty acids (and their derivative eicosanoids) and xenobiotics. PPARα has been implicated as the proposed target for the nongenotoxic (epigenetic) carcinogens, e.g., the halogenated olefin solvents

trichloroethylene and perchloroethylene. The NVS_NR_hPPARa assay used TR-FRET visualization of receptor ligand-binding, relative to a known receptor antagonist, to evaluate xenobiotic PPARα interactions. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenobiotic PPARα ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARa) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that PPARa activation in utero is the molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to impaired fertility in males (AOP Under EAGMST Review), and there is some evidence that PPARa activation is the MIE for a putative pathways leading to hepatocellular adenomas and carcinomas and may be involved in increased pancreatic acinar tumors (in mouse and rat models) (AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPARa activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

van Raalte, D. H., Li, M., Pritchard, P. H., & Wasan, K. M. (2004). Peroxisome proliferator-activated receptor (PPAR)-α: a pharmacological target with a promising future. Pharmaceutical Research, 21(9), 1531-1538. PMID: 15497675

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.969
Neutral control median absolute deviation, by plate:	0.040
Positive control well median response value, by plate:	0.143
Positive control well median absolute deviation, by plate:	0.0074
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.77
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-19.49
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPARα ligand-binding; measured using Time-resolved Fluorescent Energy Resonance Transfer (TR-FRET) between terbium-labeled anti-PPARα antibody, a fluorescent small molecule PPAR ligand tracer (Fluormone™ Pan-PPAR Green), and human PPARα ligand-binding domain.

Analytical Elements:

The NVS_NR_hPPARa assay results were analyzed as loss-of-signal in TR_FRET assays where the endpoint measured was inhibition of GW7647 binding. Raw data values were normalized as

percent of GW7647 (positive control, 100% activity). If the chemical interaction was >30% of the solvent control (DMSO) activity or at least 2 baseline median average deviations (2BMAD), the chemical was considered active against the receptor and was tested in a concentration-response assay for PPARα binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR alpha activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPARa_TRANS_up
NVS_NR_hPPARa

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.147
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.883

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for

chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen
PPAR, Peroxisome Proliferator-Activated Receptor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

29 August 2016

Date of Revisions:

22 December 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation;

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA

6. Supporting Information (existing annotations):

Assay Endpoint ID: 719

NVS_NR_hPPARg

Assay Title: NovaScreen Human Peroxisome Proliferator-activated Receptor Gamma (PPARg) Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for bioactivity. The NVS NR human PPARγ assay format allows for an efficient screening of thousands of chemicals for the ability to competitively bind to the ligand-binding domain of a xenobiotic sensing nuclear receptor. This assay was developed to screen the ToxCast chemical library for potential to interfere with peroxisome proliferator-activated receptor gamma (PPARγ) ligand-binding activity using a radiometric measurements of fluorescent polarization and a potent PPARγ receptor agonist (Ciglitazone) as a reference compound (representing 100% ligand-binding activity). Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 2 hours in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

Ligand-binding domain of human PPARγ incubated with test chemicals in 384-well microtiter plates for 2 hours prior to measuring fluorescence polarization.

Experimental System:

Ligand-binding domain of human PPARγ

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials :

Receptor Source: Human recombinant PPARγ
Reference Compound: Ciglitazone, 5nM
Method: Fluorescence Polarization

Methods:

Assay Conditions: Reactions are carried out in 50 mM Tris (pH 7.4) for 2 hours at room temperature. The fluorescence polarization signal is assessed and compared to control values in order to ascertain any interactions of test compound with the ligand binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity expected in in vivo systems. The potential for a particular compound to affect changes in PPAR signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a

measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen PPARγ ligand-binding assay used a biochemical (cell-free) platform in high-throughput (384-well microplate) format to screen the ToxCast chemical library for xenobiotic interaction with human-derived nuclear receptors. An initial screening run was conducted exposing receptors to 25 µM of each chemical (in duplicate). PPARγ ligand binding by test chemicals was analyzed using fluorescence polarization to measure ligand binding to human PPARγ by test compounds. Activity was reported as percent of Ciglitazone (a potent PPARγ agonist). If the response signal differed by over 30% or varied by a minimum of 2.0 baseline median absolute deviations (2BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding of nuclear receptor PPARγ.

Scientific Principles:

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor which regulates the expression of genes involved in fatty acid-oxidation and is a major regulator of energy homeostasis. PPARγ is primarily expressed in adipose tissue, macrophages and in the colon where it controls adipocyte differentiation, lipid storage and inflammatory responses. PPARγ agonists, the thiazolidinediones (TZDs), improve insulin sensitivity, lower glucose levels, and lower plasma triglycerides and free fatty acid (FFA) levels by enhancing their uptake into adipocytes. The NVS_NR_hPPARg assay used fluorescent polarization radiometric visualization of receptor ligand-binding, to measure modulation of PPARγ activity relative to a known receptor antagonist, to evaluate xenobiotic PPARγ interactions. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of

biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for PPARγ ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARg) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is some evidence to support a putative AOP linking PPAR gamma receptor activation with increased occurrence of sarcomas in rats, mice, and hamsters (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPAR activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Nolte, R. T., Wisely, G. B., Westin, S., Cobb, J. E., Lambert, M. H., Kurokawa, R., Rosenfeld, M. G., Willson, T. M., Glass, C. K., & Milburn, M. V. (1998). "Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ". Nature 395(6698), 137-143. (PMID: 9744270)

Assay Quality Statistics:

Neutral control well median response value, by plate:	177
Neutral control median absolute deviation, by plate:	4.45
Positive control well median response value, by plate:	56.3
Positive control well median absolute deviation, by plate:	3.78
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.76
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-24.78
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.38
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPARα ligand-binding; measured using fluorescent polarization

Analytical Elements:

The NVS_NR_hPPARg assay results were analyzed as loss-of-signal in fluorescent polarization assays. Raw data values were normalized as percent of Ciglitazone (positive control, 100% activity). If the chemical interaction was >30% of the solvent control (DMSO) activity or at least 2 baseline median average deviations (2BMAD), the chemical was considered active against the receptor and was tested in a concentration-response assay for PPARγ binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each

chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR gamma activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
ATG_PPARg_TRANS_up
OT_PPARg_PPARgSRC1_0480
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_Agonist_ratio
Tox21_PPARg_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.036
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.216

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the

latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen
PPAR, Peroxisome Proliferator-Activated Receptor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 724

NVS_NR_hTRa_Antagonist

Assay Title: NovaScreen Human Thyroid Receptor Alpha Ligand-Binding Antagonist Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for thyroidal activity. This assay format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a thyroid hormone (T3) from the ligand-binding domain of the thyroid receptor alpha, using a known thyroid receptor antagonist (Bisphenol A) as a reference compound. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 1 hour in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

Recombinant human thyroid receptor incubated in 384-well microtiter plates for 1 hour prior to measuring displacement of ligand agonist (T3) by test compounds, using AlphaLISA immunoassay to detect ligand-dependent co-factor recruitment and Bisphenol A as a TRα-binding reference compound.

Experimental System:

Recombinant human thyroid receptor nuclear protein

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: Recombinant human thyroid receptor alpha
Ligand: 3,3',5-Triiodo-L-Thyronine (T3)
Final Ligand Concentration: 15 nM
Reference Compound: Bisphenol A
Reaction: Ligand dependent binding of cofactor to the receptor.
Method: Luminescent emission at 520 – 620 nm after excitation at 680 nm.

Methods:

Incubation Conditions: Reaction is incubated for 60 minutes at room temperature in 50 mM HEPES (pH 7.4), 100 mM NaCl, 0.01% Tween-20, 0.1% Bovine Serum Albumin (fatty acid free), and 0.5 mM DTT. Acceptor and donor beads are then added and the assay is read to determine inhibition of agonist-induced binding of receptor to cofactor peptide.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity

expected in in vivo systems. The potential for a particular compound to affect changes in thyroid signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen thyroid receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (384-well microplate) format to screen the ToxCast chemical library for xenobiotic interaction with human-derived nuclear receptors. An initial screening run was conducted exposing thyroid receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using displacement of T3 relative to displacement by Bisphenol A, the positive control. If the response signal differed by over 30% or varied by a minimum of 2.0 baseline median absolute deviations (2BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding and inactivation of thyroid nuclear receptor alpha (TRα).

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting thyroid signaling pathways. TH is essential for normal brain development both before and after birth and has profound effects on cellular metabolism in almost all organs. One potential mechanism by which endocrine disrupting chemicals may produce toxic effects is by interfering with the ability of thyroid hormones (T3, triiodothyronine and T4, thyroxine) to direct normal development and metabolism. Compounds which interfere with thyroid hormone signaling can result in neurological disorders by disrupting normal developmental processes. Thyroid hormones also have important roles in the initiation and

proliferation of central nervous system and cardiovascular tissues. An important component of an endocrine disruptor screening program should be the inclusion of assays designed to screen TH disrupting compounds. The NVS_NR_hTHa_Antagonist assay used displacement of the thyroid hormone T3 (Triiodothyronine) binding to human thyroid receptor, relative to displacement by Bisphenol A, a known thyroid receptor antagonist [1, 2].

The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenobiotic ligand-binding activity.

Method Development Reference:

Moriyama, K. et al. Thyroid Hormone Action Is Disrupted by Bisphenol A as an Antagonist. J. of Clinical Endocrinology and Metabolism 87:5185 (2002)

Assay Quality Statistics:

Neutral control well median response value, by plate:	633402
Neutral control median absolute deviation, by plate:	12644.4
Positive control well median response value, by plate:	11403.5
Positive control well median absolute deviation, by plate:	372.133
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.91
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-40
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-44.6
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive binding of T3 with human thyroid receptor, relative to displacement by Bisphenol A (positive control).

Analytical Elements:

The NVS_NR_hTRa_Antagonist assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of Bisphenol A binding. Raw data values were normalized as percent of Bisphenol A (positive control, 100% inhibition) activity. If the chemical interaction was >30% of the solvent control (DMSO) activity or at least 3 baseline median average deviations (3BMAD), the chemical was considered active against the thyroid receptor and was tested in a concentration-response assay for TRα binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning

model and used in further analysis as the most appropriate predictor of xenobiotic effects. Thyroid receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRa1_TRANS_up
ATG_THRb_TRANS2_up
LTEA_HepaRG_THRSP_up
LTEA_HepaRG_THRSP_dn
Tox21_TR_LUC_GH3_Agonist
Tox21_TR_LUC_GH3_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.302
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds

recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [3].

4. Assay Documentation

4.1. References

[1] Moriyama, K., et al. (2002). J Clinical Endocrinol Metabol 87(11): 5185-5190. (PMID: 12414890)
[2] Zoeller, R. T., et al. (2005). Endocrinology 146(2): 607-612. (PMID: 15498886)
[3] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
NR, Nuclear Receptor
NVS, NovaScreen
T3, Triiodothyronine
T4, Thyroxine
TH, Thyroid Hormone
TR, Thyroid Receptor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 July 2016

Date of Revisions:

21 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Assay Endpoint ID: 725

NVS_NR_mERa

Assay Title: NovaScreen Murine Estrogen Receptor HTS Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for xenoestrogenic activity. This assay format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a radiolabeled ligand from the ligand-binding domain of the estrogen receptor. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity. This assay was run for a test duration of 18 hours in a 96-well plate.

1.2. Assay Definition

Assay Throughput:

This is a biochemical (cell-free) format, using 96-well plates to incubate radiolabeled ligand with estrogen receptor alpha for 18 hours to measure displacement of estradiol by test chemicals in a competitive binding assay.

Experimental System:

ERα nuclear protein, derived from mouse tissue

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: murine ER alpha
Radioligand: [3H] Estradiol - [1 nM]
Non-specific Determinant: 17β-Estradiol - [1 µM]
Reference Compound: 17β-Estradiol
Positive Control: 17β-Estradiol

Methods:

Incubation Conditions: Reactions are carried out in 25 mM TRIS-HCl (pH 7.4) containing 1.5 mM EDTA, 1 mM DTT, and 25 mM sodium molybdate at 0-4 °C for 18 hours. The reaction is terminated by rapid vacuum filtration onto glass fiber filters soaked in 0.5% PEI. Filters were washed with cold 50 mM NaCl. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the estrogen receptor binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective cell membrane and biotransformation capacity expected in in vivo and cell-based systems. The potential for a particular compound to affect changes in estrogen signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is

also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None Reported

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen nuclear receptor murine estrogen receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (96-well microplate) format to screen the ToxCast chemical library for xenoestrogenic interaction with murine estrogen receptors. An initial screening run was conducted exposing estrogen receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using radioligand detection (via liquid scintillation counting) of displacement of [^{3}H]-Estradiol. 17β-Estradiol (E2) was used as a positive control. If the response signal differed by over 30% from the solvent control (DMSO) or if the signal varied by more than 2.0 median average deviations (2MAD), the chemical was considered active and retested in a concentration response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding activity with estrogen nuclear receptor alpha (ERα) derived from murine tissues.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting estrogen signaling pathways. The estrogen receptor mediates gene expression in response to estrogen exposure, and modulates the activity for a wide variety of physiological processes. The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for xenoestrogenic ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor activity in early life is a molecular initiating event (MIE) leading to breast cancer in both animal and human models and to endometrial carcinoma in the mouse, and ER agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Haji, M., Kato, K., Nawata, H., & Ibayashi, H. (1981). "Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat". Brain Res 204(2), 373-386. (PMID: 6780133)

O'Keefe, J. A., & Handa, R. J. (1990). "Transient elevation of estrogen receptors in the neonatal rat hippocampus". Dev Brain Res 57(1), 119-127. (PMID: 2090365)

Assay Quality Statistics:

Neutral control well median response value, by plate:	29473
Neutral control median absolute deviation, by plate:	1040.79
Positive control well median response value, by plate:	601
Positive control well median absolute deviation, by plate:	35.58
Z' (median across all plates, using positive control wells):	0.83
SSMD (median across all plates, using positive control wells):	-19
Signal-to-noise (median across all plates, using positive control wells):	-18.65
Signal-to-background (median across all plates, using positive control wells):	0.02
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive radioligand binding of [3H] Estradiol (positive control) with estrogen receptor α obtained from murine tissue source as measured by radiometric detection.

Analytical Elements:

The NVS_NR_mER assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of [^{3}H] 17β-estradiol binding. Raw data values were normalized to DMSO (neutral control) and reported as percent of 17-β Estradiol (positive control) binding capacity. Following initial screening of test compounds at single concentration (25 µM), if the chemical response was >30% of the solvent control (DMSO) activity or at least 2 baseline median average deviations (2BMAD), the chemical was considered active against the estrogen receptor and was tested in a concentration-response assay for ER binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Estrogen receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 8
Target (nominal) number of replicates: 1
Standard minimum concentration tested: 0.02 µM
Standard maximum concentration tested: 50 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 4.33
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 25.96

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes

50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	No
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	No
599-64-4	4-Cumylphenol	Weak	Active	No
104-43-8	4-Dodecylphenol	NA	Active	No
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	No
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	Yes
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	No
77-40-7	Bisphenol B	Weak	Active	No
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	No
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	No
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	Yes
84-74-2	Dibutyl phthalate	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	Yes
56-53-1	Diethylstilbestrol	Strong	Active	No
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes

120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	No
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	No
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	No
72-33-3	Mestranol	NA	Active	No
72-43-5	Methoxychlor	Very Weak	Active	No
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	Yes
32809-16-8	Procymidone	Inactive	NA	Yes
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	14	18
Inactive	9	9

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	18	18
Inactive	5	9

In Vitro Sensitivity = 43.8%
In Vitro Specificity = 50%
Balanced Accuracy = 46.9%

In Vivo Sensitivity = 50%
In Vivo Specificity = 64.3%
Balanced Accuracy = 57.2%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and

suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
ER, Estrogen Receptor
E2, Estradiol
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 726

NVS_NR_rAR

Assay Title: NovaScreen Rat Androgen Receptor Ligand-Binding Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

High-throughput screening of in vitro chemical-target interactions across a wide variety of compounds through a broad range of biochemical interactions will help describe the chemical-assay bioactivity space for chemicals with limited available information. There exists a large number of environmental chemicals for which there is little information about the potential for xenobiotic activity. The NovaScreen NR assays utilized a cell-free platform to screen a diverse chemical library for nuclear receptor activity in a high-throughput (96-well plate) format. This format allows for an efficient screening of thousands of chemicals for the ability to bind to the receptor and displace a radiolabeled ligand from the ligand-binding domain of the androgen receptor following 18-hour incubation with test chemicals. Biochemical high-throughput screening offers preliminary evidence for chemical targets in a cell or tissues which, when combined with information from literature or targeted in vivo studies, can indicate potential pathways for toxicity.

1.2. Assay Definition

Assay Throughput:

Human androgen receptor incubated in 96-well microtiter plates for 18 hours prior to measuring displacement of R1881 radiolabeled ligand by test compounds.

Experimental System:

Rat androgen receptor recombinant LBD domain

Xenobiotic Biotransformation Potential:

None

Basic Procedure:

Materials:

Receptor Source: Testosterone pre-treated Rat prostate
Radioligand: [^{3}H]Methyltrienolone (R1881) (70-87 Ci/mmol)
Final Ligand Concentration – [1.0 nM]
Non-specific Determinant: Methyltrienolone (R1881) - [1.0 nM]
Reference Compound: Methyltrienolone (R1881)
Positive Control: Methyltrienolone (R1881)

Methods:

Incubation Conditions: Reactions are carried out in 25 mM HEPES buffer (pH 7.4) containing, 10 mM sodium molybdate, 0.5 mM DTT, 250 mM sucrose, 2.5 mM MgCl2, and 1 mM PMSF at 0-4°C for 18 hours. The reaction is terminated by rapid vacuum filtration onto GF/C filters and the radioactivity bound to the filter is compared to control values in order to ascertain any interactions of test compound with the testosterone binding site.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The NovaScreen Nuclear Receptor assays described here are run in a cell-free format, and as such lack the ability to model the protective (membrane) mechanisms and biotransformation capacity expected in in vivo systems. The potential for a particular compound to affect changes in androgen

signaling pathways is not exclusively a function of receptor-ligand binding affinity, but is also a measure of the propensity for the activated receptor-ligand complex to form dimers and recruit co-activators, of the affinity for the activated complex to bind to hormone response element DNA sequences, and of interactions with other signaling pathways.

1.3. Assay References

Assay Source Contact Information:

PerkinElmer Office
940 Winter St.
Waltham, Massachusetts 02451
United States
Tel: (781) 663-6900

Assay Publication Year:

2011

Assay Publication:

Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., & Kavlock, R. J. (2011). "Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets". Toxicology 282(1-2), 1-15. (PMID: 21251949)

Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). "Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays". Chem Res Toxicol 26(6), 878-895. (PMID: 23611293

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The NovaScreen nuclear receptor rat androgen receptor ligand-binding assay used a biochemical (cell-free) platform in high-throughput (96-well microplate) format to screen the ToxCast chemical library for xenoandrogenic interaction with rodent-derived androgen receptors. An initial screening run was conducted exposing androgen receptors to 25 µM of each chemical (in duplicate). Response to chemical perturbation was measured using radioligand detection (via liquid scintillation counting) of displacement of [^{3}H]-Methyltrienolone (R1881). R1881 was used as a positive control and compound activity was measured as loss-of-signal relative to radiolabeled R1881 AR ligand binding. If the response signal differed by over 30% or varied by a minimum of 2.0 baseline median absolute deviations (2BMAD) from the vehicle control (DMSO), the chemical was considered active and retested in a concentration–response format assay using 8 concentrations derived from a serial dilution series using half-log increments and a top concentration of 50 µM. This assay used a cell-free high-throughput format to probe a diverse chemical library for potential ligand-binding activity with androgen nuclear receptor (AR) derived from testosterone-treated rat prostrate.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are compounds which interfere with normal hormone biosynthesis, signaling or metabolism and impact regulatory pathways in humans and wildlife. Many EDCs interfere with normal steroidal activity by impacting androgen signaling pathways. The androgen receptor mediates gene expression in response to androgen exposures, and modulates the activity for a wide variety of physiological processes, particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like

developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility.

The NovaScreen assays are modifications of pre-clinical drug development assays and are designed to examine chemical effects on a broad spectrum of biochemical targets or potential molecular initiating events in a high-throughput format. This assay is designed to help identify environmental compounds with a capacity for androgen receptor ligand-binding activity.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Traish, A. M., Enzio Muller, R., & Wotiz, H. H. (1986). "Binding of 7 α, 17 α-Dimethyl-19-Nortestosterone (Mibolerone) to Androgen and Progesterone Receptors in Human and Animal Tissues". Endocrinology 118(4), 1327-1333.

Zava, D., Landrum, B., Horwitz, K., & McGuire, W. (1979). "Androgen Receptor Assay with [3H] Methyltrienolone (R1881) in the Presence of Progesterone Receptors". Endocrinology 104(4), 1007-1012.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5336
Neutral control median absolute deviation, by plate:	266.87
Positive control well median response value, by plate:	761
Positive control well median absolute deviation, by plate:	37.065
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.76
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-15.24
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Competitive radioligand binding of [^{3}H]-Methyltrienolone (R1881) (positive control) with androgen receptor obtained from rat prostrate pre-treated with testosterone.

Analytical Elements:

The NVS_NR_rAR assay results were analyzed as loss-of-signal in competitive displacement assays where the endpoint measured was inhibition of [^{3}H] Methyltrienolone (R1881) binding. Raw data values were normalized to DMSO (neutral control) and reported as percent of R1881 (positive control; calculated per plate, or as a median of positive control wells from all plates combined) binding capacity. Following initial screening of test compounds at single concentration (25 µM), if the chemical response was >30% of the solvent control (DMSO) activity or at least 3 baseline median average deviations (3BMAD), the chemical was considered active against the androgen receptor and was tested in a concentration-response assay for AR binding using 8 concentrations with 3-fold serial dilutions generally beginning at a high concentration of 50 µM. Response was calculated as a percent of positive control activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publically available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.02 µM
Standard maximum concentration tested:	50 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.172
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.035

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [1].

4. Assay Documentation

4.1. References

[1] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
DMSO, Dimethyl Sulfoxide
EDC, Endocrine disrupting chemicals
MIE, Molecular Initiating Event
NR, Nuclear Receptor
NVS, NovaScreen

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. **Potential Regulatory Applications**

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation;

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA

6. **Supporting Information (existing annotations):**

OT_AR_ARELUC_AG_1440

Assay Name: Odyssey Thera CHO-K1 Androgen Response Element Luciferase 24-hr Agonist Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera AR androgen response element (ARE) luciferase (LUC) agonist assay used CHO-K1 cells stably expressing both full-length human AR transcription factors and an ARE-luciferase reporter construct to screen a diverse chemical library for potential xenobiotic androgen receptor ligand-binding activity. This assay was developed to measure long-term transcriptional changes induced by ligand-binding of androgen receptor alpha (AR) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human AR and an ARE reporter construct driving expression of luciferase. AR interacts with androgenic ligands at the ligand-binding domain and with ARE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with cells in a 384-well plate, xenobiotic agonism of AR is detected by measuring bioluminescent signal produced by AR-moderated transcriptional activity, and xenobiotic compounds with the capacity to interfere with androgenic pathways can be quantified using a luminometer to detect an increase in bioluminescence relative to baseline activity (DMSO control).

1.2. Assay Definition

Assay Throughput:

Stably transfected CHO-K1 cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring luminescence increases resulting from AR transactivation by test compounds.

Experimental System:

CHO-K1 is an immortal mammal ovary fibroblast cell line derived from Chinese hamster cells isolated in 1957 (Puck et al. 1958). CHO-K1 is a widely used cell line with well characterized cell transfection methods frequently utilized for large-scale production of numerous pharmaceutical proteins (including hormones, antibodies, and blood factors) since these cells are capable of folding, assembling and post-translationally modifying proteins in a manner that is more comparable to humans (Kildegaard et al. 2013). The Odyssey Thera AR ARE luciferase assays used CHO-K1 cells with stably transfected full-length AR and ARE-luciferase reporter constructs.

Xenobiotic Biotransformation Potential:

CHO-K1 cells have the capacity to metabolize the anti-androgenic fungicide vinclozolin (Jacobs et al. 2008), however the intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

To identify AR agonists, CHO-K1 cells are seeded into a white-walled/white-bottom 384-well plate, followed by treatment with compounds of interest or controls for 24 hours. A luciferase assay mix containing D-luciferin and ATP in PBS is then added to the cells and luminescence quantified on a Luminoskan (Thermo Scientific) luminometer. Modulation of this assay is quantified as an increase in mean luminescence intensity relative to vehicle controls. To evaluate specificity of the AR /ARE-luc assay, several known AR agonists, AR antagonists, and ER-selective compounds were also tested in 5-pt concentration-response format in a minimum of 7 assay plates. OT determined the reproducibility of DHT-induced AR/ARE-luc transcriptional activation with a total of seven 384-well plates run on 2 different days, for 24-h time frames. A minimum of 3 replicate wells were analyzed

for each sample with 14 replicate wells for vehicle controls. Luminescence data were captured on the Luminoskan luminometer.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

Assay Publication:

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera androgen response element (ARE) luciferase (LUC) agonist assay was developed to measure long-term transcriptional changes induced by ligand-binding of androgen receptor alpha (AR) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human AR and an ARE reporter construct driving expression of luciferase. AR interacts with androgenic ligands at the ligand-binding domain and with ARE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with cells, xenobiotic agonism of AR is detected by introduction of assay mix containing D-luciferin and ATP to the culture serum, and the resultant bioluminescence can be quantified using a luminometer as an increase in mean signal relative to baseline activity (DMSO control). Concentration response models are based on 6-point concentration series (0.3 – 100 µM) run in triplicate, using 6α-Fluorotestosterone as a positive control. To determine the sensitivity of the AR/ARE-luc assay system, the range of EC50 values for DHT was determined in seven 384-well plates run on 2 different days, and values did not vary by more than 4-fold for the 24-hour endpoint.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are chemicals found in the environment or introduced in one's diet that perturb normal hormone biosynthesis, metabolism and downstream gene transcription. A significant subset of EDC's including industrial chemicals, organochlorinated pesticides, and plasticizers have the capacity to bind to the androgen receptor (AR), a member of the nuclear receptor superfamily that is activated by androgens (Luccio-Camelo and Prins 2011, Sultan et al. 2001). Due to the androgen-dependence of male sexual differentiation, exposure to

EDC's can result in reduced sperm counts, increased infertility, and elevated testicular and prostate cancer risks (Luccio-Camelo and Prins 2011).

AR is a ligand-inducible nuclear hormone receptor that mediates transcription through a series of events including ligand binding, DNA binding to androgen response elements, and interaction with various co-activators. The OT_AR_ARE_LUC_Agonist Assay uses CHOK1 cells stably transfected with full-length AR to monitor ARE-driven expression of luciferase reporter activity in response to chemical exposures over 24 hours.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.52
Neutral control median absolute deviation, by plate:	1.275
Positive control well median response value, by plate:	92.04
Positive control well median absolute deviation, by plate:	15.75
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.35
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	59.62
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	14.04
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Agonism of AR transcription factors and binding to androgen response element sequence located upstream of transfected luciferase reporter gene as measured by monitoring increased luminescent emission relative to DMSO (neutral control) baseline.

Analytical Elements:

OT_AR_ARELUC_agonist_1440 readout data was analyzed in the positive (gain of signal) fitting direction over DMSO controls (baseline), and was reported as a percentage of positive control (6α-fluorotestosterone) activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.518
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical

constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Puck, T. T., et al. (1958). J Exper Med 108(6): 945-956. (PMID: 13598821)

[2] Kildegaard, H. F., et al. (2013). Curr Opin Biotechnol 24(6): 1102-1107. (PMID: 23523260)

[3] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)

[4] Sultan, C., et al. (2001). Mol Cell Endocrinol 178(1): 99-105. (PMID: 11403899)

[5] Luccio-Camelo, D. C. and G. S. Prins (2011). J Steroid Biochem Mol Biol 127(1): 74-82. (PMID: 21515368)

[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ARE, Androgen Response Element
CHO, Chinese Hamster Ovary
EDC, Endocrine Disrupting Compounds
DBD, DNA Binding Domain
DHT, 4,5 α-Dihydrotestosterone
LBD, Ligand Binding Domain
LUC, Luciferase
MIE, Molecular Initiating Event
MTC, Maximum Tolerated Concentrations
NR, Nuclear Receptors
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PBS, Phosphate Buffered Saline
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. **Potential Regulatory Applications**

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

OT_AR_ARSRC1_0480

Assay Name: Odyssey Thera HEK293T Androgen Receptor / SRC-1 Co-activator 8-hr Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera androgen receptor/steroid receptor co-activator SRC-1 assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the androgen receptor response pathway is unimpeded. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the androgen signaling pathway following 8-hour incubation of test chemical with transformed HEK293T cells in 384-well plates

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 8 hours prior to monitoring fluorescence emission resulting from xenobiotic AR activation and co-factor recruitment.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the human AR (stably expressed in HEK293T) for xenobiotic androgen receptor activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 (Graham et al. 1977). The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid (Bylund et al. 2004). HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) (Dai et al. 2015) and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method (Lin et al. 2014).

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT AR / SRC-1 assay assessed androgen receptor chemical interactions using a rapidly maturing, intensely fluorescent mutant of YFP known as Venus, rationally dissected into two separate fragments. The fragments were obtained as follows: first, fragments coding for YFP[1] and YFP[2] (corresponding to amino acid residues 1–158 and 159–239 of the full length YFP, respectively) were generated by oligonucleotide synthesis (Blue Heron Biotechnology), and then PCR mutagenesis was used to generate the mutant fragments IFP[1] and IFP[2]. Fusion constructs were transfected into HEK293T cells with a $(Gly_4Ser)_2$ linker between the AR/SRC-1 and YFP fragment genes to facilitate complementation when interacting proteins bring fragments into close proximity. The construct is stably expressed in HEK293T cells. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free DMEM medium supplemented with 10%

dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells and subcellular compartment boundaries prior to signal detection. Images were acquired on an Evotec Opera at 2 wavelengths (488 and 635nm), and the ratio of fluorescence in the nucleus relative to fluorescence in the cytoplasm (N/C Ratio) in the 488nm channel was calculated for a minimum of 400 cells per image. Both agonists and antagonists of the AR receptor induce nuclear translocation to varying degrees.

Proprietary Elements:

Odyssey Thera assays used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera AR/SRC-1 assay is a protein-complementation assay (PCA) comprised of the full length human AR and the nuclear receptor interacting domain of SRC-1, each fused to an inactive fragment of YFP. Unliganded AR is bound by heat shock/co-chaperone proteins in an inactive state in the cytoplasm (Pratt and Toft 1997) therefore fluorescent signal in the basal or unstimulated state of the assay is predominately present in the cytoplasm. In response to ligand binding, the AR/SRC-1 YFP complex translocates from the cytoplasm to the nucleus, and this assay records compound-AR interactions by measurement of nucleus : cytoplasm (N/C) signal ratios. Each AR protein and its associated coactivator (SRC-1) contain a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the androgen responsive signaling pathway is

impacted by chemical activation or interference, the resulting YFP signal production can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic AR ligand-binding. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track changes at the level of cell functioning which may occur at a number of points along the androgen signaling pathway following an 8 hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and baseline signal and DHT (5α-Dihydrotestosterone) as a positive control and measure of 100% ligand-binding activity in AR. Concentration-response models are based on 6-point concentration series (0.3 – 100 µM) run in triplicate. Preliminary experiments examined the temporal nature of the AR/SRC-1 translocation and determined that maximum S/B was achieved after 8 hours, and while EC50s did not vary considerably over time, a more robust estimation of lower concentrations was achieved in longer duration assays. The OT AR/SRC-1 assay was also run for 16 hours (see description for OT_AR_ARSRC1_0960). OT initially treated the cells with the AR agonist 4-5, dihydrotestosterone (DHT) in 10-point concentration- response format for 8 hours to assess sensitivity of the AR/SRC-1 assay to ligand.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are chemicals found in the environment or introduced in one's diet that perturb normal hormone biosynthesis, metabolism and downstream gene transcription. A significant subset of EDC's including industrial chemicals, organochlorinated pesticides, and plasticizers have the capacity to bind to the androgen receptor (AR), a member of the nuclear receptor superfamily that is activated by androgens (Luccio-Camelo and Prins 2011, Sultan et al. 2001). Due to the androgen-dependence of male sexual differentiation, exposure to EDC's can result in reduced sperm counts, increased infertility, and elevated testicular and prostate cancer risks (Luccio-Camelo and Prins 2011).

AR is a ligand-inducible nuclear hormone receptor that mediates transcription through a series of events including ligand binding, DNA binding to androgen response elements, and interaction with various co-activators. These co-activators are components required for androgen-dependent transcription, and either physically link the AR to the basal transcriptional machinery or modulate chromatin via methylation or acetylation (McKenna et al. 1999). Over 169 proteins have been reported as potential AR co-regulators (Heemers and Tindall 2007) including the prototypical nuclear receptor coactivator, SRC-1. While numerous assays have been described in the literature that assess AR function using transcriptional readouts (Vinggaard et al. 1999), ligand competition binding (Féau et al. 2011) or cellular dynamics of GFP-tagged AR (Sultan et al. 2001, Szafran et al. 2008), the OT AR/SRC-1 assay evaluated EDC-induced AR activity in the context of the receptor's interaction with the steroid receptor co-activator protein, SRC-1. The advantage of this approach is that compounds that favor interaction of AR with SRC-1 (such as ligands), indicating the activated state of the receptor, can be readily detected. In addition, compounds that perturb this interaction by acting upstream in the pathway (e.g. through non-genomic effects) may also be identified. Therefore, this assay represents a novel tool for evaluating endocrine disrupting agents which have potential to interfere with endogenous androgen signaling in a high throughput screening mode.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a

putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.786
Neutral control median absolute deviation, by plate:	0.017
Positive control well median response value, by plate:	1.5075
Positive control well median absolute deviation, by plate:	0.0363
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.77
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	42.17
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	1.93
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor-mediated signaling pathway stable protein formation in response to AR agonism and SRC-1 co-activator recruitment measured by monitoring increased fluorescence relative to DMSO (neutral control) baseline.

Analytical Elements:

Each data point was formed by taking the log of the ratio of the sample signal to the control signal. A minimum of 8 replicate wells were analyzed each for sample and vehicle controls. Wells located in the outer ring of the plate were omitted due to the potential for edge effects. Data were captured on a Perkin Elmer Opera confocal microscope: 8 images per well in two wavelengths with a minimum of 400 cells per image. Each data point represents the average of 32 images acquired in four wells, normalized to the N/C Ratio calculated for 8 vehicle control wells (64 images). Gain-of-signal data are plotted as percent of activity where max activity corresponds to 1µM DHT, and relative to DMSO, negative control and baseline activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria;

either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.456
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds

recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals (Richard et al. 2016).

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie-Int J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Pratt, W. B. and D. O. Toft (1997). Endocr Rev 18(3): 306-360. (PMID: 9183567)
[6] Sultan, C., et al. (2001). Mol Cell Endocrinol 178(1): 99-105. (PMID: 11403899)
[7] Luccio-Camelo, D. C. and G. S. Prins (2011). J Steroid Biochem Mol 127(1): 74-82. (PMID: 21515368)
[8] McKenna, N. J., et al. (1999). J Steroid Biochem Mol Biol 69(1): 3-12. (PMID: 10418975)
[9] Heemers, H. V. and D. J. Tindall (2007). Endocr Rev 28(7): 778-808. (PMID: 17940184)
[10] Vinggaard, A. M., et al. (1999). Toxicol Appl Pharmacol 155(2): 150-160. (PMID: 10053169)
[11] Féau, C., et al. (2011). Androgen Action: Methods and Protocols: 59-68. (PMID: 19171919)
[12] Szafran, A. T., et al. (2008). PLoS One 3(11): e3605. (PMID: 18978937)
[13] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATTC, American Tissue Culture Collection
DBD, DNA Binding Domain
DHT, 4-5, Dihydrotestosterone
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA , Protein-Fragment Complementation
SRC-1, Steroid Receptor Coactivator 1
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

OT_AR_ARSRC1_0960

Assay Name: Odyssey Thera HEK293T Androgen Receptor / SRC-1 Co-activator 16-hr Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera androgen receptor/steroid receptor co-activator SRC-1 assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the androgen receptor response pathway is unimpeded. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the androgen signaling pathway following 16-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring fluorescence emission resulting from xenobiotic AR activation and co-factor recruitment.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the human AR (stably expressed in HEK293T) for xenobiotic androgen receptor activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 (Graham et al. 1977). The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid (Bylund et al. 2004). HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) (Dai et al. 2015) and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method (Lin et al. 2014).

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT AR/SRC-1 assay assessed androgen receptor chemical interactions using a rapidly maturing, intensely fluorescent mutant of YFP known as Venus, rationally dissected into two separate fragments. The fragments were obtained as follows: first, fragments coding for YFP[1] and YFP[2] (corresponding to amino acid residues 1–158 and 159–239 of the full length YFP, respectively) were generated by oligonucleotide synthesis (Blue Heron Biotechnology), and then PCR mutagenesis was used to generate the mutant fragments IFP[1] and IFP[2]. Fusion constructs were transfected into HEK293T cells with a $(Gly_4Ser)_2$ linker between the AR/SRC-1 and YFP fragment genes to facilitate complementation when interacting proteins bring fragments into close proximity. The construct is stably expressed in HEK293T cells. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free DMEM medium supplemented with 10% dextran-treated

FBS and allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 16 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells and subcellular compartment boundaries prior to signal detection. Images were acquired on an Evotec Opera at 2 wavelengths (488 and 635nm), and the ratio of fluorescence in the nucleus relative to fluorescence in the cytoplasm (N/C Ratio) in the 488nm channel was calculated for a minimum of 400 cells per image. Both agonists and antagonists of the AR receptor induce nuclear translocation to varying degrees.

Proprietary Elements:

Odyssey Thera assays used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera AR/SRC-1 assay is a protein-complementation assay (PCA) comprised of the full length human AR and the nuclear receptor interacting domain of SRC-1, each fused to an inactive fragment of YFP. Unliganded AR is bound by heat shock/co-chaperone proteins in an inactive state in the cytoplasm (Pratt and Toft 1997) therefore fluorescent signal in the basal or unstimulated state of the assay is predominately present in the cytoplasm. In response to ligand binding, the AR/SRC-1 YFP complex translocates from the cytoplasm to the nucleus, and this assay records compound-AR interactions by measurement of nucleus : cytoplasm (N/C) signal ratios. Each AR protein and its associated coactivator (SRC-1) contain a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the androgen responsive signaling pathway is

impacted by chemical activation or interference, the resulting YFP signal production can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic AR ligand-binding. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track changes at the level of cell functioning which may occur at a number of points along the androgen signaling pathway following an 16 hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and baseline signal and DHT (5α-Dihydrotestosterone) as a positive control and measure of 100% ligand-binding activity in AR. Concentration-response models are based on 6-point concentration series (0.3 – 100 µM) run in triplicate. Preliminary experiments examined the temporal nature of the AR/SRC-1 translocation and determined that maximum S/B was achieved after 8 hours, and while EC50s did not vary considerably over time, a more robust estimation of lower concentrations was achieved in longer duration assays. The OT AR/SRC-1 assay was also run for 8 hours (see description for OT_AR_ARSRC1_0480). OT initially treated the cells with the AR agonist 4-5, dihydrotestosterone (DHT) in 10-point concentration- response format for 8 hours to assess sensitivity of the AR/SRC-1 assay to ligand.

Scientific Principles:

Endocrine disrupting chemicals (EDCs) are chemicals found in the environment or introduced in one's diet that perturb normal hormone biosynthesis, metabolism and downstream gene transcription. A significant subset of EDC's including industrial chemicals, organochlorinated pesticides, and plasticizers have the capacity to bind to the androgen receptor (AR), a member of the nuclear receptor superfamily that is activated by androgens (Luccio-Camelo and Prins 2011, Sultan et al. 2001). Due to the androgen-dependence of male sexual differentiation, exposure to EDC's can result in reduced sperm counts, increased infertility, and elevated testicular and prostate cancer risks (Luccio-Camelo and Prins 2011).

AR is a ligand-inducible nuclear hormone receptor that mediates transcription through a series of events including ligand binding, DNA binding to androgen response elements, and interaction with various co-activators. These co-activators are components required for androgen-dependent transcription, and either physically link the AR to the basal transcriptional machinery or modulate chromatin via methylation or acetylation (McKenna et al. 1999). Over 169 proteins have been reported as potential AR co-regulators (Heemers and Tindall 2007) including the prototypical nuclear receptor coactivator, SRC-1. While numerous assays have been described in the literature that assess AR function using transcriptional readouts (Vinggaard et al. 1999), ligand competition binding (Féau et al. 2011) or cellular dynamics of GFP-tagged AR (Sultan et al. 2001, Szafran et al. 2008), the OT AR/SRC-1 assay evaluated EDC-induced AR activity in the context of the receptor's interaction with the steroid receptor co-activator protein, SRC-1. The advantage of this approach is that compounds that favor interaction of AR with SRC-1 (such as ligands), indicating the activated state of the receptor, can be readily detected. In addition, compounds that perturb this interaction by acting upstream in the pathway (e.g. through non-genomic effects) may also be identified. Therefore, this assay represents a novel tool for evaluating endocrine disrupting agents which have potential to interfere with endogenous androgen signaling in a high throughput screening mode.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a

putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.7645
Neutral control median absolute deviation, by plate:	0.0133
Positive control well median response value, by plate:	1.576
Positive control well median absolute deviation, by plate:	0.0341
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.82
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	21
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	60.12
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	2.05
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor-mediated signaling pathway stable protein formation in response to AR agonism and SRC-1 co-activator recruitment measured by monitoring increased fluorescence relative to DMSO (neutral control) baseline.

Analytical Elements:

Each data point was formed by taking the log of the ratio of the sample signal to the control signal. A minimum of 8 replicate wells were analyzed each for sample and vehicle controls. Wells located in the outer ring of the plate were omitted due to the potential for edge effects. Data were captured on a Perkin Elmer Opera confocal microscope: 8 images per well in two wavelengths with a minimum of 400 cells per image. Each data point represents the average of 32 images acquired in four wells, normalized to the N/C Ratio calculated for 8 vehicle control wells (64 images). Gain-of-signal data are plotted as percent of activity where max activity corresponds to 1µM DHT, and relative to DMSO, negative control and baseline activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria;

either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
Tox21_AR_BLA_Agonist_ratio
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.638
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds

recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [13].

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[6] Sultan, C., et al. (2001). Mol Cell Endocrinol 178(1): 99-105. (PMID: 11403899)
[7] Luccio-Camelo, D. C. and G. S. Prins (2011). J Steroid Biochem Mol Biol 127(1): 74-82. (PMID: 21515368)
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[9] Heemers, H. V. and D. J. Tindall (2007). Endocr Rev 28(7): 778-808. (PMID: 17940184)
[10] Vinggaard, A. M., et al. (1999). Toxicol Appl Pharmacol 155(2): 150-160.
[11] Féau, C., et al. (2011). Androgen Action: Meth Prot: 59-68. (PMID: 19171919)
[12] Szafran, A. T., et al. (2008). PLoS One 3(11): e3605. (PMID: 18978937)
[13] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
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DHT, 4-5, Dihydrotestosterone
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
SRC-1, Steroid Receptor Coactivator 1
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

OT_ER_ERaERa_0480

Assay Name: Odyssey Thera Estrogen Receptor α/α Homodimer 8-hour Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor alpha homodimer (ERα/ERα) assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the pathway is stimulated. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following 8-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 8 hour chemical exposures.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the wild type human ERα (stably expressed in HEK293T) for xenoestrogenic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT ERα / ERα assay is a homodimer PCA of the ligand binding domain (amino acids 310-547) of human ERα stably expressed in HEK293T cells. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium supplemented with 10% dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is primarily punctuate and cytoplasmic. Modulation of this assay is quantified as an increase in mean fluorescence intensity in the nucleus (for agonists) or in the nucleus and cytoplasm (antagonists) relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. The latter instrument is favored for this assay because of the rapid mode of data acquisition, the ability to acquire data from the entire cell population in a well (as opposed to

a fraction of the well on the high content devices) and the larger dynamic range typically achieved with this instrument. To assess sensitivity of the ERα/ERα LBD assay to ligand, cells were treated with the ER agonist 17-β-estradiol (E2) in 10-point concentration- response format for 8 hours and monitored response on a laser scanning plate cytometer (Acumen eX3; TTP Lab Tech).

Proprietary Elements:

Odyssey Thera assay described here used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor homodimerization, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera ERα/ERα LBD assay utilized the ability of the ERα to homodimerize upon ligand-binding with estrogenic compounds [5]. This activity is monitored via Protein-Fragment Complementation Assays (PCAs) which investigate the biochemical pathways capable of bringing separate protein fragments into close proximity. Each ERα protein contains a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the estrogenic pathway is stimulated, separate ERα proteins form homodimers and the resulting YFP signal can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic ligand-binding and ERα activation. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following an 8-hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and baseline signal and 17β-estradiol as a positive control and measure of 100% ligand-binding activity in ERα.

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [6].

Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs on reproductive development and on cell proliferation [7]. Thus, a highly sensitive assay that can detect ERα LBD binding in the context of a whole cell would serve as a powerful predictor of human-relevant estrogenic effects. The Odyssey Thera Ligand Binding assays used protein-fragment complementation (PCA) to measure dose-dependent homodimerization of estrogen receptor (ER) α expressed in human embryonic kidney cell line HEK293T. This dimerization and concurrent conformational changes brings into close proximity the fused fragments of split-yellow fluorescent protein (YFP), leading to a dramatic increase in YFP intensity. This intensity change is dose-dependent and shows saturation-binding that plateaus differently for agonists versus antagonists.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.859
Neutral control median absolute deviation, by plate:	0.256
Positive control well median response value, by plate:	15.56
Positive control well median absolute deviation, by plate:	1.10
Z' (median across all plates, using positive control wells):	0.7
SSMD (median across all plates, using positive control wells):	12
Signal-to-noise (median across all plates, using positive control wells):	53.55
Signal-to-background (median across all plates, using positive control wells):	17.85
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen receptor homodimerization in response to ligand-binding as measured with protein complementation assay technology by monitoring fluorescence intensity.

Analytical Elements:

OT_ER_ERaERa_0480 was analyzed into in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and signal baseline for activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3

Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 1.66
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	No
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	No
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes

117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	16
Inactive	14	8

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	29	13
Inactive	6	11

In Vitro Sensitivity = 56.8%
In Vitro Specificity = 36.4%
Balanced Accuracy = 46.6%

In Vivo Sensitivity = 69.0%
In Vivo Specificity = 64.7%
Balanced Accuracy = 66.9%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [8].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie- Inter J Pharma Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Katzenellenbogen, B. S., et al. (1993). J Ster Biochem Mol Biol 47(1): 39-48. (PMID: 8274440)
[6] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[7] Helguero, L. A., et al. (2005). Oncogene 24(44): 6605-6616. (PMID: 16007178)
[8] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
E2, Estradiol
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_ER_ERaERa_1440

Assay Name: Odyssey Thera Estrogen Receptor α/α Homodimer 24-hour Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor alpha homodimer (ERα/ERα) assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the pathway is stimulated. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following 24-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 24 hour chemical exposures.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the wild type human ERα (stably expressed in HEK293T) for xenoestrogenic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

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a fraction of the well on the high content devices) and the larger dynamic range typically achieved with this instrument. To assess sensitivity of the ERα/ERα LBD assay to ligand, cells were treated with the ER agonist 17-β-estradiol (E2) in 10-point concentration- response format for 24 hours and monitored response on a laser scanning plate cytometer (Acumen eX3; TTP Lab Tech).

Proprietary Elements:

Odyssey Thera assay described here used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor homodimerization, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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2003

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Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

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Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [6].

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Assay Quality Statistics:

Neutral control well median response value, by plate:	0.943
Neutral control median absolute deviation, by plate:	0.250
Positive control well median response value, by plate:	30.36
Positive control well median absolute deviation, by plate:	1.81
Z' (median across all plates, using positive control wells):	0.8
SSMD (median across all plates, using positive control wells):	17
Signal-to-noise (median across all plates, using positive control wells):	137.55
Signal-to-background (median across all plates, using positive control wells):	36.88
CV (median across all plates):	0.25

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen signaling pathway protein fragment dimerization and enzyme formation in response to estrogen receptor α ligand-binding as detected by monitoring fluorescence intensity.

Analytical Elements:

OT_ER_ERaERa_1440 was analyzed into in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and baseline activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3

Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.640
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	No
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	No
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes

117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	Yes
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	16
Inactive	14	8

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	30	12
Inactive	5	12

In Vitro Sensitivity = 56.8%
In Vitro Specificity = 36.4%
Balanced Accuracy = 46.6%

In Vivo Sensitivity = 71.4%
In Vivo Specificity = 70.6%
Balanced Accuracy =71.0%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [8].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie - Int J of Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Katzenellenbogen, B. S., et al. (1993). J Steroid Biochem Molecul Biol 47(1): 39-48. (PMID: 8274440)
[6] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[7] Helguero, L. A., et al. (2005). Oncogene 24(44): 6605-6616. (PMID: 16007178)
[8] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
E2, Estradiol
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA , Protein-Fragment Complementation
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 744

OT_ER_ERaERb_0480

Assay Name: Odyssey Thera Estrogen Receptor α/β Homodimer 8-hour Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor alpha heterodimer (ERα/ERβ) assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the estrogenic pathway is stimulated. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following 8-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 8 hour chemical exposures.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the wild type human ER (isoforms α and β, stably expressed in HEK293T cells) for xenoestrogenic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines COS-7 and HepG2 cell lines [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT ERα / ERβ assay is a heterodimer PCA of the ligand binding domains (LBD) of ERα (amino acids 310-547) and human ERβ (amino acids 263-489) stably expressed in HEK293T cells. ERα/ERβ LBD cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium supplemented with 10% dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is primarily punctuate and cytoplasmic. Modulation of this assay is quantified as an increase in mean fluorescence intensity in the nucleus (for agonists) or in the nucleus and cytoplasm (antagonists) relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. The latter instrument is favored for this assay because of the rapid mode of data acquisition, the ability to acquire data from the entire

cell population in a well (as opposed to a fraction of the well on the high content devices) and the larger dynamic range typically achieved with this instrument. To assess sensitivity of the ERα/ERβ LBD assay to ligand, cells were treated with the ER agonist 17-β-estradiol (E2) in 10-point concentration- response format for 8 hours and monitored response on a laser scanning plate cytometer (Acumen eX3; TTP Lab Tech).

Proprietary Elements:

Odyssey Thera assay described here used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor heterodimerization, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera ERα/ERβ LBD assay utilized the ability of ERα and ER to form heterodimers following ligand-binding with estrogenic compounds. This activity is monitored via Protein-Fragment Complementation Assays (PCAs) which investigate the biochemical pathways capable of bringing separate protein fragments into close proximity. Each ERα and ERβ protein contains a fragment of a reporter enzyme (YFP) and when both proteins come in contact to form homo- or heterodimers, the resulting YFP signal can be measured using fluorescence microscopy and used to screen a diverse chemical library for potential xenobiotic ligand-binding and ER activation. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following an 8 hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and

baseline signal and 17β-estradiol as a positive control and measure of 100% ligand-binding activity in ER α/β.

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [5].

The estrogen receptor is expressed in two forms, ERα and ERβ which play different roles in mediating the actions of estrogenic compounds. Multiple studies have determined that the two isoforms can form functional homo- and hetero-dimers in vitro and in vivo which are capable of binding DNA [6] and initiating transcription of target genes [7]. Furthermore, ER homo- and heterodimers display ligand-selective activity [8] leading in turn to a unique but overlapping set of dimer-mediated transcriptional changes [9, 10]. Thus, a complete understanding of the potential estrogenic effects of EDCs requires the comprehensive profiling of the three physiological dimers. To assess the activity of the ERα/β heterodimer, this assay was designed to utilize the ability of the coexpressed ERα and ERβ LBDs to heterodimerize upon ligand-binding with estrogenic compounds. This dimerization and concurrent conformational changes brings into close proximity the fused fragments of split-YFP, leading to a dramatic increase in YFP intensity. This intensity change is dose-dependent and shows saturation-binding that plateaus differently for agonists versus antagonists.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.875
Neutral control median absolute deviation, by plate:	0.126
Positive control well median response value, by plate:	3.22
Positive control well median absolute deviation, by plate:	0.21
Z' (median across all plates, using positive control wells):	0.55
SSMD (median across all plates, using positive control wells):	9
Signal-to-noise (median across all plates, using positive control wells):	19.43
Signal-to-background (median across all plates, using positive control wells):	3.64
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen signaling pathway protein fragment dimerization and enzyme formation in response to estrogen receptor (α / β) ligand-binding as detected by monitoring fluorescence intensity.

Analytical Elements:

OT_ER_ERaERb_0480 was analyzed into in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and baseline signal. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 4.66
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 23.30

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes

50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	Yes
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	25	12
Inactive	15	7

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	32	10
Inactive	8	9

In Vitro Sensitivity = 67.6%
In Vitro Specificity = 31.8%

Balanced Accuracy = 49.7%

In Vivo Sensitivity = 76.2%
In Vivo Specificity = 52.9%
Balanced Accuracy = 58.3%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [11].

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4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogen Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie-Int J Pharm Sci 70(1): 33-37. (PMID: 25975096)
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[9] Monroe, D. G., et al. (2005). Mol Endocrinol 19(6): 1555-1568. (PMID: 15802376)
[10] Li, X., et al. (2004). Mol Cell Biol 24(17): 7681-7694. (PMID: 15314175)
[11] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
E2, Estradiol
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event

NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_ER_ERaERb_1440

Assay Name: Odyssey Thera Estrogen Receptor α/β Homodimer 24-hour Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor alpha heterodimer (ERα/ERβ) assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the estrogenic pathway is stimulated. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following 24-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 24 hour chemical exposures.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the wild type human ER (isoforms α and β, stably expressed in HEK293T cells) for xenoestrogenic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT ERα / ERβ assay is a heterodimer PCA of the ligand binding domains (LBD) of ERα (amino acids 310-547) and human ERβ (amino acids 263-489) stably expressed in HEK293T cells. ERα/ERβ LBD cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium supplemented with 10% dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 24 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is primarily punctuate and cytoplasmic. Modulation of this assay is quantified as an increase in mean fluorescence intensity in the nucleus (for agonists) or in the nucleus and cytoplasm (antagonists) relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. The latter instrument is favored for this assay because of the rapid mode of data acquisition, the ability to acquire data from the entire

cell population in a well (as opposed to a fraction of the well on the high content devices) and the larger dynamic range typically achieved with this instrument. To assess sensitivity of the ERα/ERβ LBD assay to ligand, cells were treated with the ER agonist 17-β-estradiol (E2) in 10-point concentration- response format for 24 hours and monitored response on a laser scanning plate cytometer (Acumen eX3; TTP Lab Tech).

Proprietary Elements:

Odyssey Thera assay described here used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor heterodimerization, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera ERα/ERβ LBD assay utilized the ability of ERα and ER to form heterodimers following ligand-binding with estrogenic compounds. This activity is monitored via Protein-Fragment Complementation Assays (PCAs) which investigate the biochemical pathways capable of bringing separate protein fragments into close proximity. Each ERα and ERβ protein contains a fragment of a reporter enzyme (YFP) and when both proteins come in contact to form homo- or heterodimers, the resulting YFP signal can be measured using fluorescence microscopy and used to screen a diverse chemical library for potential xenobiotic ligand-binding and ER activation. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the estrogen signaling pathway following a 24 hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and

baseline signal and 17β-estradiol as a positive control and measure of 100% ligand-binding activity in ER α/β.

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [5].

The estrogen receptor is expressed in two forms, ERα and ERβ which play different roles in mediating the actions of estrogenic compounds. Multiple studies have determined that the two isoforms can form functional homo- and hetero-dimers in vitro and in vivo which are capable of binding DNA [6] and initiating transcription of target genes [7]. Furthermore, ER homo- and heterodimers display ligand-selective activity [8] leading in turn to a unique but overlapping set of dimer-mediated transcriptional changes [9, 10]. Thus, a complete understanding of the potential estrogenic effects of EDCs requires the comprehensive profiling of the three physiological dimers. To assess the activity of the ERα/β heterodimer, this assay was designed to utilize the ability of the coexpressed ERα and ERβ LBDs to heterodimerize upon ligand-binding with estrogenic compounds. This dimerization and concurrent conformational changes brings into close proximity the fused fragments of split-YFP, leading to a dramatic increase in YFP intensity. This intensity change is dose-dependent and shows saturation-binding that plateaus differently for agonists versus antagonists.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.00
Neutral control median absolute deviation, by plate:	0.140
Positive control well median response value, by plate:	6.79
Positive control well median absolute deviation, by plate:	0.43
Z' (median across all plates, using positive control wells):	0.72
SSMD (median across all plates, using positive control wells):	13
Signal-to-noise (median across all plates, using positive control wells):	43.18
Signal-to-background (median across all plates, using positive control wells):	6.82
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen signaling pathway protein fragment dimerization and enzyme formation in response to estrogen receptor (α / β) ligand-binding as detected by monitoring fluorescence intensity.

Analytical Elements:

OT_ER_ERaERb_1440 was analyzed into in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and baseline signal. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 1.91
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes

50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	Yes
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	27	10
Inactive	15	7

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	32	10
Inactive	10	7

In Vitro Sensitivity = 73.0%
In Vitro Specificity = 31.8%

Balanced Accuracy = 52.4%

In Vivo Sensitivity = 76.2%
In Vivo Specificity = 41.2%
Balanced Accuracy = 58.7%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [11].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogen Genome Res 106(1): 28-32. (PMID: 15218237)
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[9] Monroe, D. G., et al. (2005). Mol Endocrinol 19(6): 1555-1568. (PMID: 15802376)
[10] Li, X., et al. (2004). Mol Cell Biol 24(17): 7681-7694. (PMID: 15314175)
[11] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

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AOP, Adverse Outcome Pathway
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
E2, Estradiol
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event

NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA , Protein-Fragment Complementation
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_ER_ERbERb_0480

Assay Name: Odyssey Thera Estrogen Receptor β/β Homodimer 8-hour Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor beta (ERβ/ERβ) ligand-binding assay used Protein-Fragment Complementation Assays (PCAs) to probe estrogen receptor beta (ERβ) for xenoestrogenic nuclear receptor binding and subsequent homodimer formation in stably transfected human embryonic kidney cells (cell line HEK293T). This assay format is designed to investigate the biochemical pathways capable of bringing separate, rationally dissected yellow fluorescent protein (YFP) fragments, which are linked in-frame to ERβ genes, into close proximity. When the reporter enzyme fragments are physically adjacent, the result is YFP reassembly and functional signal production. This fluorescence signal is only produced when the estrogen receptor beta pathway is stimulated. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenoestrogenic impacts on whole cell functioning which may affect functioning at a number of points along the estrogen signaling pathway. This activity was monitored following 8-hour incubation of test chemical or solvent with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 8 hour chemical exposures.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the wild type human ERβ (stably expressed in HEK293T cells) for xenoestrogenic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT ERβ / ERβ assay is a homodimer PCA of the ligand binding domain (LBD; amino acids 310-547) of human ERβ stably expressed in HEK293T cells. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium supplemented with 10% dextran-treated FBS and allowed to adhere for 8 hours prior to treatment with compounds of interest or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is primarily

punctuate and cytoplasmic. Modulation of this assay is quantified as an increase in mean fluorescence intensity in the nucleus (for agonists) or in the nucleus and cytoplasm (antagonists) relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. The latter instrument is favored for this assay because of the rapid mode of data acquisition, the ability to acquire data from the entire cell population in a well (as opposed to a fraction of the well on the high content devices) and the larger dynamic range typically achieved with this instrument. To assess sensitivity of the ERβ/ERβ LBD assay to ligand, cells were treated with the ER agonist 17-β-estradiol (E2) in 10-point concentration- response format for 8 hours and monitored response on a laser scanning plate cytometer (Acumen eX3; TTP Lab Tech).

Proprietary Elements:

Odyssey Thera assay described here used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor homodimerization, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [5].

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [5].

The estrogen receptor is expressed in two forms, ERα and ERβ, which play different roles in mediating the actions of estrogenic compounds. Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs on reproductive development and on cell proliferation, while ERβ is thought to inhibit estrogen dependent cell growth, especially in breast cancer cells [6-8]. Several studies have associated a loss of ERβ or a decreased ratio of ERβ/ERα with other cancer types, including ovarian and colorectal cancers [9], suggesting a tumor suppressor role for ERβ in several cell types. Several isotype-selective ligands that bind one receptor with higher affinity than the other have been identified [10, 11] that produce distinct physiological effects when tested in animal models [9]. Therefore, a highly sensitive assay that can detect ERβ transcriptional changes in the context of a whole cell would be a useful tool to differentiate EDC's that preferentially activate ERβ-specific pathways. The OT ERβ/ERβ LBD PCA utilizes the ability of the ERβ LBD to homodimerize upon binding to estrogenic compounds. This dimizeration and concurrent conformational changes brings into close proximity the fused fragments of split-YFP, leading to a dramatic increase in YFP intensity. This intensity change is dose-dependent and shows saturation-binding that plateaus differently for agonists versus antagonists.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.11
Neutral control median absolute deviation, by plate:	0.19
Positive control well median response value, by plate:	6.75
Positive control well median absolute deviation, by plate:	0.37
Z' (median across all plates, using positive control wells):	0.7
SSMD (median across all plates, using positive control wells):	13
Signal-to-noise (median across all plates, using positive control wells):	27.9
Signal-to-background (median across all plates, using positive control wells):	5.81
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen signaling pathway protein fragment dimerization and enzyme formation in response to estrogen receptor beta ligand-binding as detected by monitoring fluorescence intensity.

Analytical Elements:

OT_ER_ERbERb_0480 assay was analyzed into in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and baseline signal. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up

NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_1440
OT_ER_ERaERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 3.04
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes

599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	Yes
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes

556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	27	10
Inactive	15	7

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	31	11
Inactive	8	9

In Vitro Sensitivity = 73.0%
In Vitro Specificity = 31.8%
Balanced Accuracy = 52.4%

In Vivo Sensitivity = 73.8%
In Vivo Specificity = 52.9%
Balanced Accuracy = 63.4%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [12].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)

[3] Dai, D., et al. (2015). Die Pharmazie- Int J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[6] Papoutsi, Z., et al. (2009). J Mol Endocrin 43(2): 65-72. (PMID: 19376833)
[7] Cowley, S. M., et al. (1997). J Biol Chem 272(32): 19858-19862. (PMID: 9242648)
[8] Powell, E. and W. Xu (2008). PNAS105(48): 19012-19017. (PMID: 19022902)
[9] Monroe, D. G., et al. (2005). Mol Endocrinol 19(6): 1555-1568. (PMID: 15802376)
[10] Li, X., et al. (2004). Mol Cell Biol 24(17): 7681-7694. (PMID: 15314175)
[11] Kraichely, D. M., et al. (2000). Endocrinology 141(10): 3534-3545. (PMID: 11014206)
[12] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
E2, Estradiol
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 747

OT_ER_ERbERb_1440

Assay Name: Odyssey Thera Estrogen Receptor β/β Homodimer 24-hour Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor beta (ERβ/ERβ) ligand-binding assay used Protein-Fragment Complementation Assays (PCAs) to probe estrogen receptor beta (ERβ) for xenoestrogenic nuclear receptor binding and subsequent homodimer formation in stably transfected human embryonic kidney cells (cell line HEK293T). This assay format is designed to investigate the biochemical pathways capable of bringing separate, rationally dissected yellow fluorescent protein (YFP) fragments which are linked in-frame to ERβ genes into close proximity. When the reporter enzyme fragments are physically adjacent, the result is YFP reassembly and functional signal production. This fluorescence signal is only produced when the estrogen receptor beta pathway is stimulated. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenoestrogenic impacts on whole cell functioning which may affect functioning at a number of points along the estrogen signaling pathway. This activity was monitored following 24-hour incubation of test chemical or solvent with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 24 hour chemical exposures.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the wild type human ERβ (stably expressed in HEK293T cells) for xenoestrogenic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT ERβ / ERβ assay is a homodimer PCA of the ligand binding domain (LBD; amino acids 310-547) of human ERβ stably expressed in HEK293T cells. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium supplemented with 10% dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 24 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is primarily

punctuate and cytoplasmic. Modulation of this assay is quantified as an increase in mean fluorescence intensity in the nucleus (for agonists) or in the nucleus and cytoplasm (antagonists) relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. The latter instrument is favored for this assay because of the rapid mode of data acquisition, the ability to acquire data from the entire cell population in a well (as opposed to a fraction of the well on the high content devices) and the larger dynamic range typically achieved with this instrument. To assess sensitivity of the ERβ/ERβ LBD assay to ligand, cells were treated with the ER agonist 17-β-estradiol (E2) in 10-point concentration- response format for 24 hours and monitored response on a laser scanning plate cytometer (Acumen eX3; TTP Lab Tech).

Proprietary Elements:

Odyssey Thera assay described here used patented PCA technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor homodimerization, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [5].

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [5].

The estrogen receptor is expressed in two forms, ERα and ERβ which play different roles in mediating the actions of estrogenic compounds. Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs on reproductive development and on cell proliferation, while ERβ is thought to inhibit estrogen dependent cell growth, especially in breast cancer cells [6-8]. Several studies have associated a loss of ERβ or a decreased ratio of ERβ/ERα with other cancer types, including ovarian and colorectal cancers [9], suggesting a tumor suppressor role for ERβ in several cell types. Several isotype-selective ligands that bind one receptor with higher affinity than the other have been identified [10, 11] that produce distinct physiological effects when tested in animal models [9]. Therefore, a highly sensitive assay that can detect ERβ transcriptional changes in the context of a whole cell would be a useful tool to differentiate EDC's that preferentially activate ERβ-specific pathways. The OT ERβ/ERβ LBD PCA utilizes the ability of the ERβ LBD to homodimerize upon binding to estrogenic compounds. This dimizeration and concurrent conformational changes brings into close proximity the fused fragments of split-YFP, leading to a dramatic increase in YFP intensity. This intensity change is dose-dependent and shows saturation-binding that plateaus differently for agonists versus antagonists.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.25
Neutral control median absolute deviation, by plate:	0.19
Positive control well median response value, by plate:	14.01
Positive control well median absolute deviation, by plate:	0.65
Z' (median across all plates, using positive control wells):	0.79
SSMD (median across all plates, using positive control wells):	18
Signal-to-noise (median across all plates, using positive control wells):	67.22
Signal-to-background (median across all plates, using positive control wells):	10.89
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Estrogen signaling pathway protein fragment dimerization and enzyme formation in response to estrogen receptor beta ligand-binding as detected by monitoring fluorescence intensity.

Analytical Elements:

OT_ER_ERbERb_1440 was analyzed into in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and baseline signal. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa

OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_1440
OT_ER_ERaERb_0480
OT_ER_ERbERb_0480
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 1.48
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No

104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	No
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No

57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	24	13
Inactive	13	9

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	29	13
Inactive	8	9

In Vitro Sensitivity = 64.9%
In Vitro Specificity = 40.9%
Balanced Accuracy = 52.9%

In Vivo Sensitivity = 69.0%
In Vivo Specificity = 52.9%
Balanced Accuracy = 61.0%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [12].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogen Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie-Int J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[6] Papoutsi, Z., et al. (2009). J Molecul Endocrin 43(2): 65-72. (PMID: 19376833)

[7] Cowley, S. M., et al. (1997). J Biol Chem 272(32): 19858-19862. (PMID: 9242648)
[8] Powell, E. and W. Xu (2008). PNAS 105(48): 19012-19017. (PMID: 19022902)
[9] Monroe, D. G., et al. (2005). Mol Endocrinol 19(6): 1555-1568. (PMID: 15802376)
[10] Li, X., et al. (2004). Mol Cell Biol 24(17): 7681-7694. (PMID: 15314175)
[11] Kraichely, D. M., et al. (2000). Endocrinology 141(10): 3534-3545. (PMID: 11014206)
[12] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
E2, Estradiol
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 May2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_ERa_EREGFP_0120

Assay Name: Odyssey Thera HeLa cell-based high content GFP:Prolactin 2-hour assay to monitor active Estrogen Receptor (ER) transcriptional units

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor alpha (ERα) GFP estrogen response element (ERE) assay used human cervical adenocarcinoma (HeLa) cells stably expressing both full-length, fluorescently-tagged human ERα transcription factor and multiple tandem prolactin promotor sequences to screen a diverse chemical library for potential xenoestrogenic activity. Sensitive microscopic visualization of prolactin promoter occupancy by a GFP-tagged ERα was capable of measuring agonist- and antagonist- ERα-ligand binding following a 2-hour incubation of test compounds with cells in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

HeLa cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to treatment with test compounds or controls for 2 hours.

Experimental System:

GFP_ERα:PRL-HeLa cells are cervical adenocarcinoma epithelial cells, isolated from a 31 year old African-American female in February, 1951 [1], which constitutively express fluorescently-tagged full length human ERα and multiple integrated prolactin promotor sequences. The stable ER:PRL-HeLa cell line was developed by the Mancini lab at Baylor College of Medicine [2].

Xenobiotic Biotransformation Potential:

Constitutive expression of CYP1A1 and CYP1B1 mRNA; CYP1A2 expression was examined but not detected in HeLa cells [3, 4]. Expression of tumor-suppressing p53 and pRB proteins has been reported to be low [5].

Basic Procedure:

The stable ERα:PRL-HeLa line is seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium with 10% dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with test compounds or controls for 2 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. All assay endpoints are quantified using high content image analysis algorithms.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource-intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2014

Assay Publication:

Stossi, F., Bolt, M. J., Ashcroft, F. J., Lamerdin, J. E., Melnick, J. S., Powell, R. T., Dandekar, R. D., Mancini, M. G., Walker, C. L., Westwick, J. K., & Mancini, M. A. (2014). "Defining estrogenic mechanisms of bisphenol A analogs through high throughput microscopy-based contextual assays". Chem Biol 21(6), 743-753. (PMID: 24856822)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

This Odyssey Thera estrogen receptor α green fluorescent protein (GFP) estrogen response element (ERE) assay was developed to measure transcriptional changes induced by ligand-binding as detected in a cervical adenocarcinoma cell line stably expressing both full-length human ERα and multiple estrogen responsive prolactin promoter sequences. ERα interacts with estrogenic ligands and following 2-hour incubation of test compound with cells in a 384-well plate, xenoestrogenic activation of a microscopically visible reporter gene is detected as an increase in mean signal relative to baseline activity (DMSO control) using a 10-point concentration-response assay format.

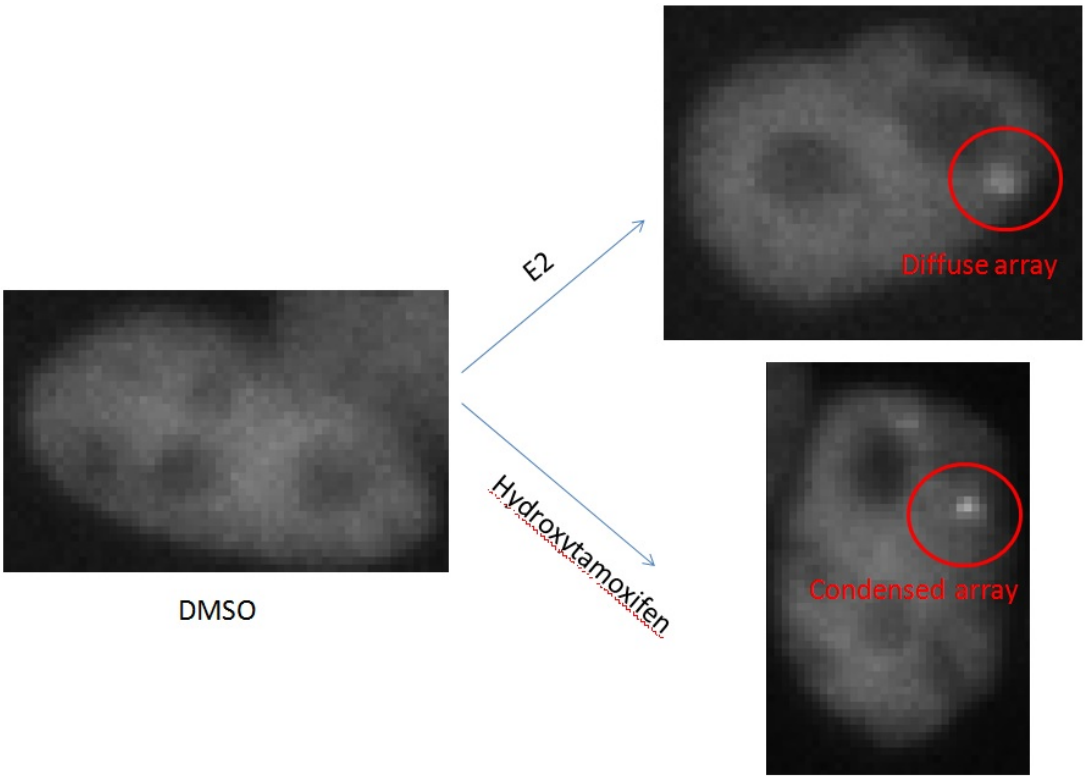

Figure 1. Single-cell views of the ER:PRL-HeLa cell line showing GFP-ERα accumulation on ERE arrays in response to vehicle (DMSO), agonist (E2) or antagonist (4-hydroxytamoxifen). Images were taken on an InCell 2000 with a 20X 0.75NA objective, provided curtesy of Odyssey Thera, Inc.

The ER:PRL-HeLa line constitutively expresses physiologically-relevant levels of fluorescently-tagged, full-length human ERα, and contains multi-copy genomic insertions of the prolactin promoter containg estrogen receptor response elements. When stimulated by agonists, tagged ERα accumulates on the prolactin array in an open (transcriptionally-active) binding mode, leading to a bright, micron-sized spot while antagonist-treated cells lead to tagged-ERα binding in its closed (transcriptionally-repressive) binding-mode, leading to a condensed array that appears as a sub-micron-sized point.

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD's proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [6]. Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males. Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Ashcroft, F. J., Newberg, J. Y., Jones, E. D., Mikic, I., & Mancini, M. A. (2011). "High content imaging-based assay to classify estrogen receptor-α ligands based on defined mechanistic outcomes". Gene 477(1-2), 42-52. (PMID: 21256200)

Sharp, Z. D., Mancini, M. G., Hinojos, C. A., Dai, F., Berno, V., Szafran, A. T., et al. (2006). Estrogen-receptor-α exchange and chromatin dynamics are ligand-and domain-dependent. Journal of cell science, 119(19), 4101-4116. (PMID: 16968748)

Assay Quality Statistics:

Neutral control well median response value, by plate:	8.75
Neutral control median absolute deviation, by plate:	1.48
Positive control well median response value, by plate:	321.5
Positive control well median absolute deviation, by plate:	16.49
Z' (median across all plates, using positive control wells):	0.81
SSMD (median across all plates, using positive control wells):	19
Signal-to-noise (median across all plates, using positive control wells):	129.09
Signal-to-background (median across all plates, using positive control wells):	35.68
CV (median across all plates):	0.17

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Ligand binding of estrogen receptor α and xenoestrogenic effects on transcriptional regulation of a fluorescent reporter gene.

Analytical Elements:

OT_ERa_GFPERaERE_0120 readout data was analyzed in the positive (gain of signal) fitting direction using percent activity 4-Hydroxytamoxifen as positive control (100% activity) over DMSO controls. All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor transactivation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM

Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.488
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	No
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	No
480-40-0	Chrysin	Very Weak	NA	No
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes

115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	Yes
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	22	15
Inactive	14	8

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	29	13
Inactive	7	10

In Vitro Sensitivity = 59.5%
In Vitro Specificity = 36.4%
Balanced Accuracy = 48.0%

In Vivo Sensitivity = 69.0%
In Vivo Specificity = 64.7%
Balanced Accuracy = 58.8%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [7].

4. Assay Documentation

4.1. References

[1] Jones, H. W., et al. (1971). Obstetrics & Gynecology 38(6): 945-949. (PMID: 4942173)
[2] Nakajima, M., et al. (2003). Toxicol Lett 144(2): 247-256. (PMID: 12927368)
[3] Iwanari, M., et al. (2002). Arch Toxicol 76(5-6): 287-298. (PMID: 12107646)
[4] Scheffner, M., et al. (1991). PNAS 88(13): 5523-5527. (PMID: 1648218)
[5] Ashcroft, F. J., et al. (2011). Gene 477(1-2): 42-52. (PMID: 21256200)
[6] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[7] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

4HT, 4-hydroxytamoxifen
AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathways
DMSO, Dimethyl Sulfoxide
E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
GFP, Green Fluorescent Protein
DBD, DNA Binding Domain
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
MTC, Maximum Tolerated Concentrations
NR, Nuclear Receptors
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PBS, Phosphate Buffered Saline
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 751

OT_ERa_EREGFP_0480

Assay Name: Odyssey Thera HeLa cell-based high content GFP:Prolactin 8-hour assay to monitor active Estrogen Receptor (ER) transcriptional units

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen receptor alpha (ERα) GFP estrogen response element (ERE) assay used human cervical adenocarcinoma (HeLa) cells stably expressing both full-length, fluorescently-tagged human ERα transcription factor and multiple tandem prolactin promotor sequences to screen a diverse chemical library for potential xenoestrogenic activity. Sensitive microscopic visualization of prolactin promoter occupancy by a GFP-tagged ERα was capable of measuring agonist- and antagonist- ERα-ligand binding following a 8-hour incubation of test compounds with cells in a 384-well plate.

1.2. Assay Definition

Assay Throughput:

HeLa cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to treatment with test compounds or controls for 8 hours.

Experimental System:

GFP_ERα:PRL-HeLa cells are cervical adenocarcinoma epithelial cells, isolated from a 31 year old African-American female in February, 1951 [1], which constitutively express fluorescently-tagged full length human ERα and multiple integrated prolactin promotor sequences.

Xenobiotic Biotransformation Potential:

Constitutive expression of CYP1A1 and CYP1B1 mRNA; CYP1A2 expression was examined but not detected in HeLa cells [2, 3]. Expression of tumor-suppressing p53 and pRB proteins has been reported to be low [4].

Basic Procedure:

The stable ERα:PRL-HeLa line is seeded into optical quality 384-well poly-D-lysine coated plates in phenol red-free medium with 10% dextran-treated FBS and allowed to adhere for 24 hours prior to treatment with test compounds or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells prior to signal detection. All assay endpoints are quantified using high content image analysis algorithms.

Proprietary Elements:

This assay is not proprietary. ER:PRL-HeLa cell line was developed and provided to OT by the Mancini lab at Baylor [5].

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2014

Assay Publication:

Stossi, F., Bolt, M. J., Ashcroft, F. J., Lamerdin, J. E., Melnick, J. S., Powell, R. T., Dandekar, R. D., Mancini, M. G., Walker, C. L., Westwick, J. K., & Mancini, M. A. (2014). "Defining estrogenic mechanisms of bisphenol A analogs through high throughput microscopy-based contextual assays". Chem Biol 21(6), 743-753. (PMID: 24856822)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

This Odyssey Thera estrogen receptor α green fluorescent protein (GFP) estrogen response element (ERE) assay was developed to measure long-term transcriptional changes induced by ligand-binding as detected in a cervical adenocarcinoma cell line stably expressing both full-length human ERα and multiple estrogen responsive prolactin promoter sequences. ERα interacts with estrogenic ligands and following 2-hour incubation of test compound with cells in a 384-well plate, xenoestrogenic activation of a microscopically visible reporter gene is detected as an increase in mean signal relative to baseline activity (DMSO control) using a 10-point concentration-response assay format.

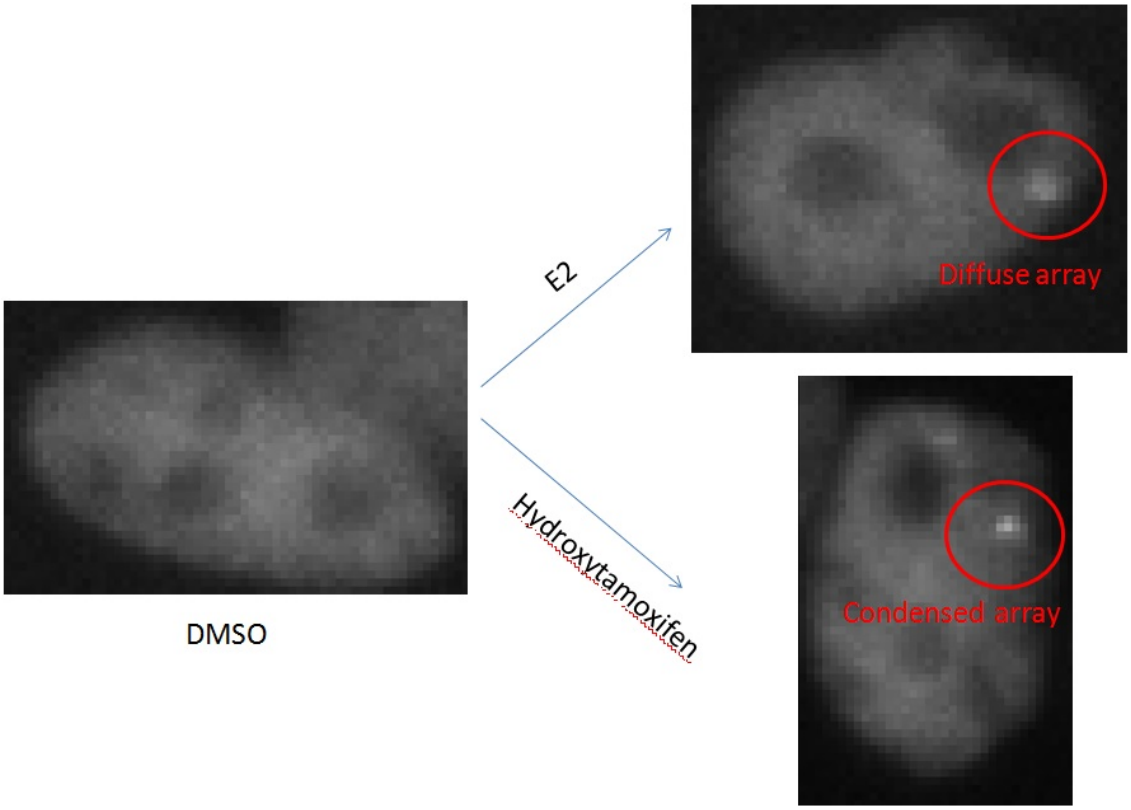

Figure 1. Single-cell views of the ER:PRL-HeLa cell line showing GFP-ERα accumulation on ERE arrays in response to vehicle (DMSO), agonist (E2) or antagonist (4-hydroxytamoxifen). Images were taken on an InCell 2000 with a 20X 0.75NA objective, provided curtesy of Odyssey Thera, Inc.

ER:PRL-HeLa line constitutively expresses physiologically-relevant levels of fluorescently-tagged, full-length human ERα, and contains multi-copy genomic insertions of the prolactin promoter containg estrogen receptor response elements. When stimulated by agonists, tagged ERα accumulates on the prolactin array in an open (transcriptionally-active) binding mode, leading to a bright, micron-sized spot while antagonist-treated cells lead to tagged-ERα binding in its closed (transcriptionally-repressive) binding-mode, leading to a condensed array that appears as a sub-micron-sized point).

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NHRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD's proclivity to bind a

disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [6]. Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the MIE leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative adverse outcome pathways leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males. Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Ashcroft, F. J., Newberg, J. Y., Jones, E. D., Mikic, I., & Mancini, M. A. (2011). "High content imaging-based assay to classify estrogen receptor-α ligands based on defined mechanistic outcomes". Gene 477(1-2), 42-52. (PMID: 21256200)

Sharp, Z. D., Mancini, M. G., Hinojos, C. A., Dai, F., Berno, V., Szafran, A. T., et al. (2006). Estrogen-receptor-α exchange and chromatin dynamics are ligand-and domain-dependent. Journal of cell science, 119(19), 4101-4116. (PMID: 16968748)

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.5
Neutral control median absolute deviation, by plate:	0.741
Positive control well median response value, by plate:	351.25
Positive control well median absolute deviation, by plate:	16.68
Z' (median across all plates, using positive control wells):	0.84
SSMD (median across all plates, using positive control wells):	20
Signal-to-noise (median across all plates, using positive control wells):	410.09
Signal-to-background (median across all plates, using positive control wells):	52.13
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Ligand binding of estrogen receptor α and xenoestrogenic effects on transcriptional regulation of a fluorescent reporter gene.

Analytical Elements:

OT_ERa_GFPERaERE_0480 readout data was analyzed in the positive (gain of signal) fitting direction using percent activity 4-Hydroxytamoxifen as positive control (100% activity) over DMSO controls. All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the

baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 6
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.3 µM
Standard maximum concentration tested: 100 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.227
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	No

131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	No
104-40-5	4-Nonylphenol	Very Weak	Active	No
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	No
61-82-5	Amitrole	NA	Inactive	No
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	No
480-40-0	Chrysin	Very Weak	NA	No
50-22-6	Corticosterone	Inactive	NA	No
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	Yes
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	No
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	Yes
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes

51630-58-1	Fenvalerate	NA	Inactive	No
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	No
520-18-3	Kaempferol	Very Weak	Inactive	No
143-50-0	Kepone	Weak	NA	Yes
65277-42-1	Ketoconazole	Inactive	NA	Yes
330-55-2	Linuron	Inactive	NA	No
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	No
72-55-9	p,p'-DDE	Very Weak	Weak	No
87-86-5	Pentachlorophenol	NA	Inactive	No
57-30-7	Phenobarbital sodium	Inactive	NA	No
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	Yes
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	16
Inactive	13	9

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	26	16
Inactive	8	9

In Vitro Sensitivity = 56.8%
In Vitro Specificity = 40.9%
Balanced Accuracy = 48.9%

In Vivo Sensitivity = 61.9%
In Vivo Specificity = 52.9%
Balanced Accuracy = 57.4%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for

chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [7].

4. Assay Documentation

4.1. References

[1] Jones, H. W., et al. (1971). Obstetrics & Gynecology 38(6): 945-949. (PMID: 4942173)
[2] Nakajima, M., et al. (2003). Toxicol Lett 144(2): 247-256. (PMID: 12927368)
[3] Iwanari, M., et al. (2002). Arch Toxicol 76(5-6): 287-298. (PMID: 12107646)
[4] Scheffner, M., et al. (1991). PNAS 88(13): 5523-5527. (PMID: 1648218)
[5] Ashcroft, F. J., et al. (2011). Gene 477(1-2): 42-52. (PMID: 21256200)
[6] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[7] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

4HT, 4-hydroxytamoxifen
AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathways
DMSO, Dimethyl Sulfoxide
E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
GFP, Green Fluorescent Protein
DBD, DNA Binding Domain
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event
MTC, Maximum Tolerated Concentrations
NR, Nuclear Receptors
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PBS, Phosphate Buffered Saline
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_ERa_ERELUC_AG_1440

Assay Name: Odyssey Thera CHO-K1 cell-based luciferase assay to measure estrogen receptor alpha / ERE transcriptional activity (agonist mode)

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen response element (ERE) luciferase (LUC) agonist assay was developed to measure long-term transcriptional changes induced by ligand-binding of estrogen receptor alpha (ERα) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human ERα and an ERE reporter construct driving expression of luciferase. ERα interacts with estrogenic ligands at the ligand-binding domain and with ERE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with cells in a 384-well plate, xenobiotic agonism of ERα is detected by measuring bioluminescent signal produced by ER-moderated transcriptional activity, and potential xenoestrogenic compounds can be identified using a luminometer to quantify an increase in mean signal relative to baseline activity (DMSO control).

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 24 hour chemical exposures.

Experimental System:

CHO-K1 is an immortal mammal ovary fibroblast cell line derived from Chinese hamster cells isolated in 1957 [1]. CHO-K1 is a widely used cell line with well characterized cell transfection methods frequently utilized for large-scale production of numerous pharmaceutical proteins (including hormones, antibodies, and blood factors) since these cells are capable of folding, assembling and post-translationally modifying proteins in a manner that is more comparable to humans [2]. The Odyssey Thera ERE_luc assays used CHO-K1 cells with stably transfected full-length ERα and ERE-luciferase reporter construct.

Xenobiotic Biotransformation Potential:

CHO-K1 cells have the capacity to metabolize the anti-androgenic fungicide vinclozolin [3], however the intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

To identify ER agonists, CHO-K1 cells are seeded into a white-walled/white-bottom 384-well plate followed by treatment with compounds of interest or controls for 24 hours. A luciferase assay mix containing D-luciferin and ATP in PBS is then added to the cells and luminescence quantified on a Luminoskan (Thermo Scientific) luminometer. Modulation of this assay is quantified as an increase in mean luminescence intensity relative to vehicle controls.

To evaluate specificity of the ERα /ERE-luc assay, several known ER agonists were also tested in 5-pt concentration-response format in a minimum of 7 assay plates.

OT determined the reproducibility of E2-induced ERα/ERE-luc transcriptional activation with a total of seven 384-well plates run on 3 different days, for 24 hours. A minimum of 3 replicate wells were analyzed for each sample with 14 replicate wells for vehicle controls. Luminescence data were

captured on the Luminoskan luminometer. The range of EC50 values for E2 was determined in the ERα/ERE-luc transcriptional assay in 9 384-well plates run on 3 different days.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

Assay Publication:

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera estrogen response element (ERE) luciferase (LUC) agonist assay was developed to measure transcriptional changes induced by ligand-binding of estrogen receptor alpha (ERα) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human ERα and an ERE reporter construct driving expression of luciferase. ERα interacts with estrogenic ligands at the ligand-binding domain and with ERE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with cells in a 384-well plate, xenobiotic agonism of ERα is detected by measuring bioluminescent signal produced by ER-moderated transcriptional activity, and potential xenoestrogenic compounds can be identified using a luminometer to quantify an increase in mean signal relative to baseline activity (DMSO control).

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NHRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [4].

Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs on reproductive development and on cell proliferation [5]. Thus, a highly sensitive assay that can detect ERα LBD binding in the context of a whole cell would serve as a

powerful predictor of human-relevant estrogenic effects. The OT_ERa_ERE_LUC_Agonist Assay uses CHOK1 cells stably transfected with full-length ERα to monitor ERE-driven expression of luciferase reporter activity in response to chemical exposures over 24 hours.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds which potentially affect endocrine systems in exposed populations by interacting with estrogen receptor mediated signaling pathways. There is evidence that estrogen receptor activation in early life is a molecular initiating event (MIE) in a developing Adverse Outcome Pathways (AOP) leading to endometrial carcinoma in the mouse (currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of ER interference in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Katzenellenbogen, B. S., Bhardwaj, B., Fang, H., Ince, B. A., Pakde, F., Reese, J. C., Schodin, D., & Wrenn, C. K. (1993). "Hormone binding and transcription activation by estrogen receptors: analyses using mammalian and yeast systems". The Journal of Steroid Biochemistry and Molecular Biology 47(1), 39-48. (PMID: 8274440)

Assay Quality Statistics:

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Agonism of ERα transcription factor and DNA-binding to ERE sequence located upstream of transfected luciferase reporter gene measured by monitoring increased luminescence.

Analytical Elements:

OT_ERa_ERE_LUC_agonist_1440 readout data was analyzed in the positive fitting direction (receptor gain-of-signal activity) as a percent of 17b-Estradiol (positive control, 100% activation) and relative to DMSO, negative control and baseline activity. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up

ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_1440
OT_ER_ERaERb_0480
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Puck, T. T., et al. (1958). The J Exper Medicine 108(6): 945-956. (PMID: 13598821)
[2] Kildegaard, H. F., et al. (2013). Curr Opin Biotechnol 24(6): 1102-1107. (PMID: 23523260)
[3] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[4] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)

[5] Helguero, L. A., et al. (2005). Oncogene 24(44): 6605-6616. (PMID: 16007178)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
CHO, Chinese Hamster Ovary
DMSO, Dimethyl Sulfoxide
E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
DBD, DNA Binding Domain
LBD, Ligand Binding Domain
LUC, Luciferase
MIE, Molecular Initiating Event
MTC, Maximum Tolerated Concentrations
NR, Nuclear Receptors
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PBS, Phosphate Buffered Saline
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 May 2016

Date of Revisions:

25 November 2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 749

OT_ERa_ERELUC_ANT_1440

Assay Name: Odyssey Thera CHO-K1 cell-based luciferase assay to measure estrogen receptor alpha / ERE transcriptional activity (antagonist mode)

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen response element (ERE) luciferase (LUC) antagonist assay was developed to measure transcriptional changes induced by ligand-binding of estrogen receptor alpha (ERα) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human ERα and an ERE reporter construct driving expression of luciferase. ERα interacts with estrogenic ligands at the ligand-binding domain and with ERE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with cells in a 384-well plate, xenobiotic antagonism of ERα is detected by measuring a reduction in bioluminescent signal produced by ER-moderated transcriptional activity induced by a known ER agonist (17beta-estradiol), and potential estrogenic compounds can be identified using a luminometer to quantify an increase in activity relative to vehicle controls (DMSO).

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 24 hour chemical exposures.

Experimental System:

CHO-K1 is an immortal mammal ovary fibroblast cell line derived from Chinese hamster cells isolated in 1957 [1]. CHO-K1 is a widely used cell line with well characterized cell transfection methods frequently utilized for large-scale production of numerous pharmaceutical proteins (including hormones, antibodies, and blood factors) since these cells are capable of folding, assembling and post-translationally modifying proteins in a manner that is more comparable to humans [2]. The Odyssey Thera ERE_luc assays used CHO-K1 cells with stably transfected full-length ERα and ERE-luciferase reporter construct.

Xenobiotic Biotransformation Potential:

CHO-K1 cells have the capacity to metabolize the anti-androgenic fungicide vinclozolin [3], however the intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

To identify ER antagonists, the ERα/ERE-luc assay is run in the presence of low amounts of ER ligand. The cells are seeded into a white-walled/white-bottom 384-well plate in the presence of agonist (1nM 17β-Estradiol), followed by treatment with compounds of interest or controls for 24 hours. A luciferase assay mix containing D-luciferin and ATP in PBS is then added to the cells and luminescence quantified on a Luminoskan (Thermo Scientific) luminometer. Modulation of this assay is quantified as an increase in mean luminescence intensity relative to vehicle controls. To demonstrate sensitivity of the ERα /ERE-luc assay to ER antagonists, OT treated the cells with 4-hydroxytamoxifen (4HT) in concentration-response format for 24 hours in the presence of E2. To evaluate specificity of the ERα /ERE-luc assay, several known ER agonists, ER antagonists, and AR-selective compounds were also tested in 5-pt concentration-response format in a minimum of 7 assay plates. OT determined the reproducibility of 4HT-mediated inhibition of E2-stimulated ERα/ERE-Luciferase transcriptional activation with a total of nine 384-well plates run on 3 different

days, and EC50 values for 4HT were determined at the 24h time point. A minimum of 3 replicate wells were analyzed for each sample with 14 replicate wells for vehicle controls. Luminescence data were captured on the Luminoskan luminometer.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

Assay Publication:

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera estrogen response element (ERE) luciferase (LUC) antagonist assay was developed to measure transcriptional changes induced by ligand-binding of estrogen receptor alpha (ERα) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human ERα and an ERE reporter construct driving expression of luciferase. ERα interacts with estrogenic ligands at the ligand-binding domain and with ERE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with agonist-stimulated cells in a 384-well plate, xenobiotic antagonism of ERα is detected by measuring a reduction in bioluminescent signal induced by a known ER agonist (17beta-estradiol) relative to vehicle controls (DMSO).

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [4].

Cell-based and in vivo experiments suggest that the ERα isoform is the primary mediator of estrogenic effects by EDCs on reproductive development and on cell proliferation [5]. Thus, a sensitive assay that can detect ERα LBD binding in the context of a whole cell would serve as a

powerful predictor of human-relevant estrogenic effects. The OT_ERa_ERE_LUC_Antagonist Assay uses CHO-K1 cells stably transfected with full-length ERα to monitor xenobiotic interference with ERE-driven expression of luciferase reporter activity in response to chemical exposures over 24 hours.

Method Development Reference:

Katzenellenbogen, B. S., Bhardwaj, B., Fang, H., Ince, B. A., Pakde, F., Reese, J. C., Schodin, D., & Wrenn, C. K. (1993). "Hormone binding and transcription activation by estrogen receptors: analyses using mammalian and yeast systems". The Journal of steroid biochemistry and molecular biology 47(1), 39-48. (PMID: 8274440)

Assay Quality Statistics:

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Antagonism of ERα transcription factor and repression of DNA-binding to ERE sequence located upstream of transfected luciferase reporter gene measured by monitoring increased luminescence.

Analytical Elements:

OT_ERa_ERE_LUC_Antagonist_1440 readout data was analyzed in the positive fitting direction using log2 fold-induction over DMSO controls. Activity was monitored as loss-of-signal relative to the positive control of (Z)-4-Hydroxytamoxifen. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_1440
OT_ER_ERaERb_0480

OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERa_ERELUC_AG_1440
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Puck, T. T., et al. (1958). The J Exper Medicine 108(6): 945-956. (PMID: 13598821)
[2] Kildegaard, H. F., et al. (2013). Curr Opin Biotechnol 24(6): 1102-1107. (PMID: 23523260)
[3] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[4] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[5] Helguero, L. A., et al. (2005). Oncogene 24(44): 6605-6616. (PMID: 16007178)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

4HT, 4-hydroxytamoxifen
AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
CHO, Chinese Hamster Ovary
DMSO, Dimethyl Sulfoxide

E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
DBD, DNA Binding Domain
LBD, Ligand Binding Domain
LUC, Luciferase
MIE, Molecular Initiating Event
MTC, Maximum Tolerated Concentrations
NR, Nuclear Receptors
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PBS, Phosphate Buffered Saline
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_ERb_ERELUC_ANT_1440

Assay Name: Odyssey Thera CHO-K1 cell-based luciferase assay to measure estrogen receptor beta / ERE transcriptional activity (antagonist mode)

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera estrogen response element (ERE) luciferase (LUC) antagonist assay was developed to measure transcriptional changes induced by ligand-binding of estrogen receptor beta (ERβ) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human ERβ and an ERE reporter construct driving expression of luciferase. ERβ interacts with estrogenic ligands at the ligand-binding domain and with ERE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with cells in a 384-well plate, xenobiotic antagonism of ERβ is detected by measuring a reduction in bioluminescent signal produced by ER-moderated transcriptional activity induced by a known ER agonist (17beta-estradiol), and potential estrogenic compounds can be identified using a luminometer to quantify an increase in activity relative to vehicle controls (DMSO).

1.2. Assay Definition

Assay Throughput:

HEK-293T cells are seeded into 384-well microtiter plates and allowed to adhere for 24 hours prior to 24 hour chemical exposures.

Experimental System:

CHO-K1 is an immortal mammal ovary fibroblast cell line derived from Chinese hamster cells isolated in 1957 [1]. CHO-K1 is a widely used cell line with well characterized cell transfection methods frequently utilized for large-scale production of numerous pharmaceutical proteins (including hormones, antibodies, and blood factors) since these cells are capable of folding, assembling and post-translationally modifying proteins in a manner that is more comparable to humans [2]. The Odyssey Thera ERE_luc assays used CHO-K1 cells with stably transfected full-length ER and ERE-luciferase reporter construct.

Xenobiotic Biotransformation Potential:

CHO-K1 cells have the capacity to metabolize the anti-androgenic fungicide vinclozolin [3], however the intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

To identify ER antagonists, the ERβ/ERE-luc assay is run in the presence of low amounts of ER ligand. The cells are seeded into a white-walled/white-bottom 384-well plate in the presence of agonist (1nM 17β-Estradiol), followed by treatment with compounds of interest or controls for 24 hours. A luciferase assay mix containing D-luciferin and ATP in PBS is then added to the cells and luminescence quantified on a Luminoskan (Thermo Scientific) luminometer. Modulation of this assay is quantified as an increase in mean luminescence intensity relative to vehicle controls. To demonstrate sensitivity of the ERβ/ERE-luc assay to ER antagonists, cells were treated with 4-hydroxytamoxifen (4HT) in concentration-response format for 24 hours in the presence of E2. OT determined the reproducibility of 4HT-mediated inhibition of E2-stimulated ERβ/ERE-Luciferase transcriptional activation with a total of three 384-well plates run on a single day, and EC_{50} values for 4HT were determined from those plates at the 24h time point. A minimum of 3 replicate wells

were analyzed for each sample with 14 replicate wells for vehicle controls. Luminescence data were captured on the Luminoskan luminometer.

Proprietary Elements:

This assay is not proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

Assay Publication:

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Odyssey Thera estrogen response element (ERE) luciferase (LUC) antagonist assay was developed to measure transcriptional changes induced by ligand-binding of estrogen receptor alpha (ER□) detected in a mammalian (Chinese hamster ovary; CHO-K1) cell line stably expressing both full-length human ER□ and an ERE reporter construct driving expression of luciferase. ER□ interacts with estrogenic ligands at the ligand-binding domain and with ERE sequences at the DNA-binding domain. Following 24-hour incubation of compound of interest with agonist-stimulated cells in a 384-well plate, xenobiotic antagonism of ER□ is detected by measuring a reduction in bioluminescent signal induced by a known ER agonist (17beta-estradiol) relative to vehicle controls (DMSO).

Scientific Principles:

Endocrine disrupting compounds bind to nuclear hormone receptors, leading to a diverse array of transcriptional and signaling pathway alterations. These cell- and tissue-type specific changes affect many aspects of human physiology, including those involved in inflammation, neonatal development and oncogenesis. Among the human NHRs, the estrogen receptor family is particularly susceptible to perturbation by EDCs because of the ER LBD proclivity to bind a disparate set of small hydrophobic molecules commonly found in nature and in man-made materials [4].

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds which potentially affect endocrine systems in exposed populations by interacting with estrogen receptor mediated signaling pathways. Chemical-activity profiles derived from this assay can inform prioritization decisions for compound

selection in more resource intensive *in vivo* studies to further investigate the involvement of ER interference in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Katzenellenbogen, B. S., Bhardwaj, B., Fang, H., Ince, B. A., Pakde, F., Reese, J. C., Schodin, D., & Wrenn, C. K. (1993). "Hormone binding and transcription activation by estrogen receptors: analyses using mammalian and yeast systems". The Journal of Steroid Biochemistry and Molecular Biology 47(1), 39-48. (PMID: 8274440)

Assay Quality Statistics:

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Antagonism of ERβ transcription factor and DNA-binding to ERE sequence located upstream of transfected luciferase reporter gene measured by monitoring increased luminescence.

Analytical Elements:

OT_ERβ_ERE_LUC_Antagonist_1440 readout data was analyzed in the positive fitting direction using log2 fold-induction over DMSO controls. Activity was monitored as loss-of-signal relative to the positive control of (Z)-4-Hydroxytamoxifen (100% inhibition). All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_1440
OT_ER_ERaERb_0480
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440

OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERa_ERELUC_ANT_1440
OT_ERa_ERELUC_AG_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	ZZZBB
Target (nominal) number of replicates:	ZZZCB
Standard minimum concentration tested:	ZZZDB µM
Standard maximum concentration tested:	ZZZEB µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	ZZZFB
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	ZZZGB

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Puck, T. T., et al. (1958). J Exper Med 108(6): 945-956. (PMID: 13598821)
[2] Kildegaard, H. F., et al. (2013). Curr Opin Biotechnol 24(6): 1102-1107. (PMID: 23523260)
[3] Jacobs, M., et al. (2008). Curr Drug Metab 9(8): 796-826. (PMID: 18855613)
[4] Shanle, E. K. and W. Xu (2010). Chem Res Toxicol 24(1): 6-19. (PMID: 21053929)
[5] Helguero, L. A., et al. (2005). Oncogene 24(44): 6605-6616. (PMID: 16007178)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
CHO, Chinese Hamster Ovary
DMSO, Dimethyl Sulfoxide
E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
DBD, DNA Binding Domain
LBD, Ligand Binding Domain
LUC, Luciferase
MIE, Molecular Initiating Event
MTC, Maximum Tolerated Concentrations
NR, Nuclear Receptors
NHR, Nuclear Hormone Receptors
OT, Odyssey Thera
PBS, Phosphate Buffered Saline
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

26 May 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

OT_FXR_FXRSRC1_0480

Assay Name: Odyssey Thera Farnesoid X Receptor (FXR) / Steroid Receptor Co-Activator 8-hr Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera Farnesoid X Receptor/Steroid Receptor Co-Activator SRC-1 assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the farnesoid X receptor (FXR; NR1H4) response pathway is unimpeded. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the FXR signaling pathway. The Odyssey Thera FXR/SRC-1 assay is a transient PCA performed in HEK293T cells expressing the full length human FXR and the nuclear receptor interacting domain (NID) of SRC-1, each fused to a fragment of YFP. The plasmids are co-expressed for 24 hours prior to 8-hour incubation with test compounds in 384-well plates.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 8 hours prior to monitoring fluorescence emission resulting from xenobiotic FXR activation and co-factor recruitment.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the human FXR protein (transiently expressed in HEK293T) for xenobiotic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT FXR/SRC-1 assay assessed receptor-chemical interactions using a rapidly maturing, intensely fluorescent mutant of YFP known as Venus, rationally dissected into two separate fragments. The fragments were obtained as follows: first, fragments coding for YFP[1] and YFP[2] (corresponding to amino acid residues 1–158 and 159–239 of the full length YFP, respectively) were generated by oligonucleotide synthesis (Blue Heron Biotechnology), and then PCR mutagenesis was used to generate the mutant fragments IFP[1] and IFP[2]. Fusion constructs were transfected into HEK293T cells with a $(Gly_4Ser)_2$ linker between the FXR/SRC-1 and YFP fragment genes to

facilitate complementation when interacting proteins bring fragments into close proximity. The construct is transiently transfected in HEK293T cells 24 hours prior to treatment with test compounds. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in MEM alpha medium (Invitrogen) supplemented with 10% FBS (Gemini Bio-Products) 1% penicillin and 1% streptomycin, and grown in 37 °C incubator equilibrated to 5% CO2. Cells are allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells and subcellular compartment boundaries prior to signal detection. Fluorescent signal in the basal state of the assay is restricted to the nucleus. Modulation of this assay is quantified as an increase in mean fluorescence in the nucleus relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. 8 Images per well were acquired on an Evotec Opera at 2 wavelengths (488 and 635nm), and the mean nuclear fluorescence in the 488nm channel was calculated for a minimum of 400 cells per image.

Proprietary Elements:

Odyssey Thera PCA assays used patented technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote FXR mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The OT FXR/SRC-1 assay is a PCA expressed transiently in HEK293T cells for 24h prior to treatment with test compound for 8 hours. Cells are fixed with 4% formaldehyde and stained with Draq5 to identify cells and subcellular compartment boundaries prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is restricted to the nucleus. Modulation of this assay

is quantified as an increase in mean fluorescence in the nucleus relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. Stimulation of the assay was assessed in a 10-point dose response with the selective FXR agonist GW4064 with a concentration range of 10 - 0.0003µM. Each FXR protein and its associated coactivator (SRC-1) contain a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the FXR signaling pathway is impacted by chemical activation or interference, the resulting YFP signal production can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic ligand-binding. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track changes at the level of cell functioning which may occur at a number of points along the signaling pathway following an 8-hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and baseline signal and GW4064 as a positive control and measure of 100% FXR/SRC-1 activation.

Scientific Principles:

The farnesoid X receptor (FXR) is a nuclear receptor whose primary function is to act as a bile acid sensor, protecting the liver from bile acid toxicity by regulating the transcription of genes involved in bile acid homeostasis [5]. Recent studies have also established that FXR is a master regulator of lipid and glucose homeostasis [6] and disruption of FXR in mouse models leads to phenotypes associated with metabolic disease, including diabetes and hypercholesterolemia [6]. FXR also plays roles in liver regeneration [7]. FXR is a non-steroidal nuclear receptor that binds to DNA as a heterodimer with the retinoic acid receptor, RXRα. The FXR/RXR heterodimer is "permissive" in that the pair becomes transcriptionally active in the presence of either an RXR-selective ligand or a FXR ligand [8]. This heterodimer is thought to be pre-bound to the target response element on DNA prior to ligand binding, existing in a complex with corepressor proteins. Upon ligand binding, changes in the conformation of FXR result in release of the corepressors and subsequent binding to transcriptional coactivator proteins. FXR binds to a number of co-activator proteins, including the prototypical nuclear receptor coactivator, SRC-1 [9]. Since FXR/RXRα complexes are not expected to be selective for FXR activation while ligand binding favors interaction of FXR with SRC-1, this assay is designed to monitor the interaction between FXR and SRC-1 to detect xenobiotic activation of FXR by test compounds.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.423
Neutral control median absolute deviation, by plate:	3.87
Positive control well median response value, by plate:	104.77
Positive control well median absolute deviation, by plate:	8.521
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.46
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	17.81
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	2.81

Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

farnesoid X receptor signaling pathway protein formation in response FXR ligand-binding and FXR/SRC-1 protein complex formation.

Analytical Elements:

Each data point was formed by taking the log of the ratio of the sample signal to the vehicle control (DMSO) signal. A minimum of 8 replicate wells were analyzed each for sample and vehicle controls. Wells located in the outer ring of the plate were omitted due to the potential for edge effects. Data were captured on a confocal microscope: the fold increase in mean fluorescence intensity was calculated relative to the vehicle controls from 16 images, each containing a minimum of 350 cells. Gain-of-signal activity indicates FXR/SRC-1 complex formation and data are plotted as percent of GW4064 activity, and are plotted relative to DMSO, negative control and signal baseline. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up
ATG_FXR_TRANS_up
NVS_NR_hFXR_Agonist
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.013
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.064

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharm Intern J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Commun 5. (PMID: 25182477)
[5] Kim, I., et al. (2007). J Lipid Res 48(12): 2664-2672. (PMID: 17720959)
[6] Ma, K., et al. (2006). The Journal of Clin Invest 116(4): 1102-1109. (PMID: 16557297)
[7] Huang, W., et al. (2006). Science 312(5771): 233-236. (PMID: 16614213)
[8] Pérez, E., et al. (2012). Biochimica et Biophysica Acta (1): 57-69. (PMID: 21515403)
[9] Wang, Z., et al. (2006). Cell Metabolism 3(2): 111-122. (PMID: 16459312)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATTC, American Tissue Culture Collection
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
FXR, Farnesoid X Receptor
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
SRC-1, Steroid Receptor Coactivator 1

YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 October 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

OT_FXR_FXRSRC1_1440

Assay Name: Odyssey Thera Farnesoid X Receptor (FXR) / Steroid Receptor Co-Activator 24-hr Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera Farnesoid X Receptor/Steroid Receptor Co-activator SRC-1 assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the farnesoid X receptor (FXR; NR1H4) response pathway is unimpeded. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the FXR signaling pathway. The Odyssey Thera FXR/SRC-1 assay is a transient PCA performed in HEK293T cells expressing the full length human FXR and the nuclear receptor interacting domain (NID) of SRC-1, each fused to a fragment of YFP. The plasmids are co-expressed for 24 hours prior to 24 hour incubation with test compounds in 384-well plates.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 8 hours prior to monitoring fluorescence emission resulting from xenobiotic FXR activation and co-factor recruitment.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the human FXR protein (transiently expressed in HEK293T) for xenobiotic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT FXR/SRC-1 assay assessed receptor-chemical interactions using a rapidly maturing, intensely fluorescent mutant of YFP known as Venus, rationally dissected into two separate fragments. The fragments were obtained as follows: first, fragments coding for YFP[1] and YFP[2] (corresponding to amino acid residues 1–158 and 159–239 of the full length YFP, respectively) were generated by oligonucleotide synthesis (Blue Heron Biotechnology), and then PCR mutagenesis was used to generate the mutant fragments IFP[1] and IFP[2]. Fusion constructs were transfected into HEK293T cells with a $(Gly_4Ser)_2$ linker between the FXR/SRC-1 and YFP fragment genes to

facilitate complementation when interacting proteins bring fragments into close proximity. The construct is transiently transfected in HEK293T cells 24 hours prior to treatment with test compounds. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in MEM alpha medium (Invitrogen) supplemented with 10% FBS (Gemini Bio-Products) 1% penicillin and 1% streptomycin, and grown in 37 °C incubator equilibrated to 5% CO2. Cells are allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 24 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells and subcellular compartment boundaries prior to signal detection. Fluorescent signal in the basal state of the assay is restricted to the nucleus. Modulation of this assay is quantified as an increase in mean fluorescence in the nucleus relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. 8 Images per well were acquired on an Evotec Opera at 2 wavelengths (488 and 635nm), and the mean nuclear fluorescence in the 488nm channel was calculated for a minimum of 400 cells per image.

Proprietary Elements:

Odyssey Thera PCA assays used patented technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote FXR mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None Reported

2. Assay Component Descriptions

Assay Objectives:

The OT FXR/SRC-1 assay is a PCA expressed transiently in HEK293T cells for 24h prior to treatment with test compound for 8 hours. Cells are fixed with 4% formaldehyde and stained with Draq5 to identify cells and subcellular compartment boundaries prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is restricted to the nucleus. Modulation of this assay

is quantified as an increase in mean fluorescence in the nucleus relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. Stimulation of the assay was assessed in a 10-point dose response with the selective FXR agonist GW4064 with a concentration range of 10 - 0.0003µM. Each FXR protein and its associated coactivator (SRC-1) contain a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the FXR signaling pathway is impacted by chemical activation or interference, the resulting YFP signal production can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic ligand-binding. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track changes at the level of cell functioning which may occur at a number of points along the signaling pathway following a 24-hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and baseline signal and GW4064 as a positive control and measure of 100% FXR/SRC-1 activation.

Scientific Principles:

The farnesoid X receptor (FXR) is a nuclear receptor whose primary function is to act as a bile acid sensor, protecting the liver from bile acid toxicity by regulating the transcription of genes involved in bile acid homeostasis [5]. Recent studies have also established that FXR is a master regulator of lipid and glucose homeostasis [6] and disruption of FXR in mouse models leads to phenotypes associated with metabolic disease, including diabetes and hypercholesterolemia [6]. FXR also plays roles in liver regeneration [7]. FXR is a non-steroidal nuclear receptor that binds to DNA as a heterodimer with the retinoic acid receptor, RXRα. The FXR/RXR heterodimer is "permissive" in that the pair becomes transcriptionally active in the presence of either an RXR-selective ligand or a FXR ligand [8]. This heterodimer is thought to be pre-bound to the target response element on DNA prior to ligand binding, existing in a complex with corepressor proteins. Upon ligand binding, changes in the conformation of FXR result in release of the corepressors and subsequent binding to transcriptional coactivator proteins. FXR binds to a number of co-activator proteins, including the prototypical nuclear receptor coactivator, SRC-1 [9]. Since FXR/RXRα complexes are not expected to be selective for FXR activation while ligand binding favors interaction of FXR with SRC-1, this assay is designed to monitor the interaction between FXR and SRC-1 to detect xenobiotic activation of FXR by test compounds.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	72.869
Neutral control median absolute deviation, by plate:	8.851
Positive control well median response value, by plate:	179.36
Positive control well median absolute deviation, by plate:	12.127
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.33
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	11.05
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	2.36

Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Farnesoid X receptor signaling pathway protein formation in response to FXR/SRC-1 protein complex formation following ligand-binding.

Analytical Elements:

Each data point was formed by taking the log of the ratio of the sample signal to the vehicle control (DMSO) signal. A minimum of 8 replicate wells were analyzed each for sample and vehicle controls. Wells located in the outer ring of the plate were omitted due to the potential for edge effects. Data were captured on a confocal microscope: the fold increase in mean fluorescence intensity was calculated relative to the vehicle controls from 16 images, each containing a minimum of 350 cells. Gain-of-signal activity indicates FXR/SRC-1 complex formation and data are plotted as percent of GW4064 activity, and are plotted relative to DMSO, negative control and signal baseline. package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up
ATG_FXR_TRANS_up
NVS_NR_hFXR_Agonist
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.324
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	41.62

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [10].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharm Intern J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Commun 5. (PMID: 25182477)
[5] Kim, I., et al. (2007). J Lipid Res 48(12): 2664-2672. (PMID: 17720959)
[6] Ma, K., et al. (2006). The Journal of Clin Invest 116(4): 1102-1109. (PMID: 16557297)
[7] Huang, W., et al. (2006). Science 312(5771): 233-236. (PMID: 16614213)
[8] Pérez, E., et al. (2012). Biochimica et Biophysica Acta (1): 57-69. (PMID: 21515403)
[9] Wang, Z., et al. (2006). Cell Metabolism 3(2): 111-122. (PMID: 16459312)
[10] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
ATTC, American Tissue Culture Collection
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
FXR, Farnesoid X Receptor
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
OT, Odyssey Thera
PCA, Protein-Fragment Complementation
SRC-1, Steroid Receptor Coactivator 1

YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2 October 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

OT_PPARg_PPARgSRC1_0480

Assay Name: Odyssey Thera Peroxisome Proliferator-activated Receptor Gamma (PPARg) / SRC-1 Co-activator 8-hr Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera peroxisome proliferator-activated receptor gamma/steroid receptor co-activator SRC-1 assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the peroxisome proliferator-activated receptors (PPAR) response pathway is unimpeded. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the PPAR signaling pathway following 8-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 8 hours prior to monitoring fluorescence emission resulting from xenobiotic PPAR gamma activation and co-factor recruitment.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the human PPARγ protein (transiently expressed in HEK293T) for xenobiotic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT PPARγ/SRC-1 assay assessed receptor-chemical interactions using a rapidly maturing, intensely fluorescent mutant of YFP known as Venus, rationally dissected into two separate fragments. The fragments were obtained as follows: first, fragments coding for YFP[1] and YFP[2] (corresponding to amino acid residues 1–158 and 159–239 of the full length YFP, respectively) were generated by oligonucleotide synthesis (Blue Heron Biotechnology), and then PCR mutagenesis was used to generate the mutant fragments IFP[1] and IFP[2]. Fusion constructs were transfected into HEK293T cells with a $(Gly_4Ser)_2$ linker between the PPARγ/SRC-1 and YFP fragment genes to facilitate complementation when interacting proteins bring fragments into close proximity. The construct is transiently transfected in HEK293T cells 48 hours prior to treatment with test

compounds. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in MEM alpha medium (Invitrogen) supplemented with 10% FBS (Gemini Bio-Products) 1% penicillin and 1% streptomycin, and grown in 37°C incubator equilibrated to 5% CO2. Cells are allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 8 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells and subcellular compartment boundaries prior to signal detection. 8 Images per well were acquired on an Evotec Opera at 2 wavelengths (488 and 635nm), and the ratio of fluorescence in the nucleus relative to fluorescence in the cytoplasm (N/C Ratio) in the 488nm channel was calculated for a minimum of 350 cells per image.

Proprietary Elements:

Odyssey Thera PCA assays used patented technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote PPAR gamma mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The OT PPARγ/SRC-1 assay is a PCA expressed transiently in HEK293T cells for 48h prior to treatment with test compound for 8 hours. Cells are fixed with 4% formaldehyde and stained with Draq5 to identify cells and subcellular compartment boundaries prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is restricted to the nucleus. Modulation of this assay is quantified as an increase in mean fluorescence in the nucleus relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. Stimulation of the assay was assessed in a 10-point dose response with the selective

PPARγ agonist GW1929. Each PPARγ protein and its associated coactivator (SRC-1) contain a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the PPARγ responsive signaling pathway is impacted by chemical activation or interference, the resulting YFP signal production can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic PPARγ ligand-binding. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track changes at the level of cell functioning which may occur at a number of points along the signaling pathway following an 8-hour incubation of cells with test compound in 384-well plate, using DMSO as a negative control and baseline signal and GW1929 as a positive control and measure of 100% PPARγ/SRC1 activation.

Scientific Principles:

The peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor that plays a key role in mediating differentiation of adipocytes and regulating fat metabolism. PPARγ has been implicated in the pathophysiology of atherosclerosis, inflammation, obesity, diabetes, immune response, and ageing. The PPARγ nuclear receptor functions as a transcription factor as part of a large protein complex through interactions with transcriptional co-repressors and co-activators. PPARs form heterodimers with the retinoid X receptor (RXR) and bind to PPAR response elements (PPREs) in enhancer sites of regulated genes. In the absence of ligand, nuclear receptor co-repressors bind to these heterodimers and recruit histone deactylases (HDACs) to repress transcription. Ligand binding to the C-terminal activation function (AF-2) domain induces a conformational change in the receptor dimer which excludes co-repressors from the complex. Ligand binding also increases PPAR's affinity for a number of co-activators, including SRC-1, whose binding facilitates chromatin remodeling by histone modification and nucleosome mobilization, leading to the recruitment of the basal transcription machinery to PPAR target genes. Therefore, measuring stimulation of the PPARγ/SRC-1 complex represents ligand-dependent activation of the receptor.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARg) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is some evidence to support a putative AOP linking PPAR gamma receptor activation with increased occurrence of sarcomas in rats, mice, and hamsters (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPAR activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	11.16
Neutral control median absolute deviation, by plate:	1.825
Positive control well median response value, by plate:	107.941
Positive control well median absolute deviation, by plate:	7.784
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.69

Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	60.83
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	10.57
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Peroxisome proliferator-activated receptor gamma signaling pathway protein formation in response to PPARγ ligand-binding /SRC-1 co-factor recruitment.

Analytical Elements:

Each data point was formed by taking the log of the ratio of the sample signal to the vehicle control (DMSO) signal. A minimum of 8 replicate wells were analyzed each for sample and vehicle controls. Wells located in the outer ring of the plate were omitted due to the potential for edge effects. Data were captured on a confocal microscope: the fold increase in mean fluorescence intensity was calculated relative to the vehicle controls from 16 images, each containing a minimum of 350 cells. Gain-of-signal activity indicates PPARγ/SRC-1 complex formation and data are plotted as percent of GW1929 activity, and are plotted relative to DMSO, negative control and signal baseline. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR gamma activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
ATG_PPARg_TRANS_up
NVS_NR_hPPARg
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_Agonist_ratio
Tox21_PPARg_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.192
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [5].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie Int J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATTC, American Tissue Culture Collection
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event

OT, Odyssey Thera
PCA, Protein-Fragment Complementation
PPAR, Peroxisome Proliferator-Activated Receptor
SRC-1, Steroid Receptor Coactivator 1
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

22 December 2016

Author of Revisions:

EPA NCCT

5. Supporting Information

OT_PPARg_PPARgSRC1_1440

Assay Name: Odyssey Thera Peroxisome Proliferator-activated Receptor Gamma (PPARg) / SRC-1 Co-activator 24-hr Protein-Complementation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The Odyssey Thera peroxisome proliferator-activated receptor gamma/steroid receptor co-activator SRC-1 assay used Protein-Fragment Complementation Assays (PCAs) to investigate the biochemical pathways which bring separate protein fragments into close proximity and result in functional reporter enzyme (yellow fluorescent protein, YFP) signal production when the peroxisome proliferator-activated receptors (PPAR) response pathway is unimpeded. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track xenobiotic changes at the level of cell functioning which may occur at a number of points along the PPAR signaling pathway following 24-hour incubation of test chemical with transformed HEK293T cells in 384-well plates.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 384-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from xenobiotic PPAR gamma activation and co-factor recruitment.

Experimental System:

This assay monitors the ligand binding domain (LBD) of the human PPARγ protein (transiently expressed in HEK293T) for xenobiotic activation. The HEK-293 cell line are human embryonic epithelial kidney cells (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK-293T cells are derived from the HEK293 cell line by the addition of the SV40 large T antigen that has been shown to increase vector production of some viral vectors. HEK293T are reported to have relatively high transfection efficiencies when compared to other cell lines (COS-7 and HepG2) [3] and it is among the most frequently utilized cell lines for in small-scale protein production and in viral vector propagation using the transient transfection method [4].

Xenobiotic Biotransformation Potential:

Constitutive expression of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

The OT PPARγ/SRC-1 assay assessed receptor-chemical interactions using a rapidly maturing, intensely fluorescent mutant of YFP known as Venus, rationally dissected into two separate fragments. The fragments were obtained as follows: first, fragments coding for YFP[1] and YFP[2] (corresponding to amino acid residues 1–158 and 159–239 of the full length YFP, respectively) were generated by oligonucleotide synthesis (Blue Heron Biotechnology), and then PCR mutagenesis was used to generate the mutant fragments IFP[1] and IFP[2]. Fusion constructs were transfected into HEK293T cells with a $(Gly_4Ser)_2$ linker between the PPARγ/SRC-1 and YFP fragment genes to facilitate complementation when interacting proteins bring fragments into close proximity. The construct is transiently transfected in HEK293T cells 48 hours prior to treatment with test

compounds. Cells are seeded into optical quality 384-well poly-D-lysine coated plates in MEM alpha medium (Invitrogen) supplemented with 10% FBS (Gemini Bio-Products) 1% penicillin and 1% streptomycin, and grown in 37°C incubator equilibrated to 5% CO2. Cells are allowed to adhere for 24 hours prior to treatment with compounds of interest or controls for 24 hours. Cells are fixed in 4% formaldehyde and stained with Draq5 (BioStatus) to identify cells and subcellular compartment boundaries prior to signal detection. 8 Images per well were acquired on an Evotec Opera at 2 wavelengths (488 and 635nm), and the ratio of fluorescence in the nucleus relative to fluorescence in the cytoplasm (N/C Ratio) in the 488nm channel was calculated for a minimum of 350 cells per image.

Proprietary Elements:

Odyssey Thera PCA assays used patented technology.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote PPAR gamma mediated DNA transcription, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

Odyssey Thera Inc.
4550 Norris Canyon Road, Suite 140
San Ramon, CA 94583
Tel: (925) 242-5000

Assay Publication Year:

2003

Assay Publication:

MacDonald, M. L., Lamerdin, J., Owens, S., Keon, B. H., Bilter, G. K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S. W., & Westwick, J. K. (2006). "Identifying off-target effects and hidden phenotypes of drugs in human cells". Nat Chem Biol 2(6), 329-337. (PMID: 16680159)

Yu, H., West, M., Keon, B. H., Bilter, G. K., Owens, S., Lamerdin, J., & Westwick, J. K. (2003). "Measuring drug action in the cellular context using protein-fragment complementation assays". Assay Drug Dev Technol 1(6), 811-822. (PMID: 15090227)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The OT PPARγ/SRC-1 assay is a PCA expressed transiently in HEK293T cells for 48h prior to treatment with test compounds for 24 hours. Cells are fixed with 4% formaldehyde and stained with Draq5 to identify cells and subcellular compartment boundaries prior to signal detection. Fluorescent signal in the basal state of the assay is very low and is restricted to the nucleus. Modulation of this assay is quantified as an increase in mean fluorescence in the nucleus relative to vehicle controls, and can be quantified on a high content imaging device or a laser scanning cytometer. Stimulation of the assay was assessed in a 10-point dose response with the selective

PPARγ agonist GW1929. Each PPARγ protein and its associated coactivator (SRC-1) contain a rationally dissected fragment of a yellow-fluorescent protein (YFP) reporter enzyme. When the PPARγ responsive signaling pathway is impacted by chemical activation or interference, the resulting YFP signal production can be measured using fluorescence microscopy to screen a diverse chemical library for potential xenobiotic PPARγ ligand-binding. Changes in protein complex interactions can be impacted by a variety of biochemical events within a pathway, and this assay is designed to track changes at the level of cell functioning which may occur at a number of points along the signaling pathway following a 24-hour incubation of cells with test chemicals in 384-well plate, using DMSO as a negative control and baseline signal and GW1929 as a positive control and measure of 100% PPARγ/SRC1 activation.

Scientific Principles:

The peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor that plays a key role in mediating differentiation of adipocytes and regulating fat metabolism. PPARγ has been implicated in the pathophysiology of atherosclerosis, inflammation, obesity, diabetes, immune response, and ageing. The PPARγ nuclear receptor functions as a transcription factor as part of a large protein complex through interactions with transcriptional co-repressors and co-activators. PPARs form heterodimers with the retinoid X receptor (RXR) and bind to PPAR response elements (PPREs) in enhancer sites of regulated genes. In the absence of ligand, nuclear receptor co-repressors bind to these heterodimers and recruit histone deactylases (HDACs) to repress transcription. Ligand binding to the C-terminal activation function (AF-2) domain induces a conformational change in the receptor dimer which excludes co-repressors from the complex. Ligand binding also increases PPAR's affinity for a number of co-activators, including SRC-1, whose binding facilitates chromatin remodeling by histone modification and nucleosome mobilization, leading to the recruitment of the basal transcription machinery to PPAR target genes. Therefore, measuring stimulation of the PPARγ/SRC-1 complex represents ligand-dependent activation of the receptor.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with peroxisome proliferator-activated receptor alpha (PPARg) receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is some evidence to support a putative AOP linking PPAR gamma receptor activation with increased occurrence of sarcomas in rats, mice, and hamsters (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of PPAR activation in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Michnick, S. W., Remy, I., Campbell-Valois, F. X., Vallee-Belisle, A., & Pelletier, J. N. (2000). "Detection of protein-protein interactions by protein fragment complementation strategies". Methods Enzymol 328, 208-230. (PMID: 11075347)

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.104
Neutral control median absolute deviation, by plate:	1.483
Positive control well median response value, by plate:	133.452
Positive control well median absolute deviation, by plate:	11.684
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.68

Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	90.3
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	14.2
Signal-to-background (median across all plates, using positive control wells):	NA
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Peroxisome proliferator-activated receptor gamma signaling pathway protein formation in response to PPARγ ligand-binding /SRC-1 co-factor recruitment.

Analytical Elements:

Each data point was formed by taking the log of the ratio of the sample signal to the vehicle control (DMSO) signal. A minimum of 8 replicate wells were analyzed each for sample and vehicle controls. Wells located in the outer ring of the plate were omitted due to the potential for edge effects. Data were captured on a confocal microscope: the fold increase in mean fluorescence intensity was calculated relative to the vehicle controls from 16 images, each containing a minimum of 350 cells. Gain-of-signal activity indicates PPARγ/SRC-1 complex formation and data are plotted as percent of GW1929 activity, and are plotted relative to DMSO, negative control and signal baseline. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR activity was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 30% of neutral controls or 3 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
ATG_PPARg_TRANS_up
NVS_NR_hPPARg
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_Agonist_ratio
Tox21_PPARg_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.938
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [5].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Dai, D., et al. (2015). Die Pharmazie Int J Pharm Sci 70(1): 33-37. (PMID: 25975096)
[4] Lin, Y.-C., et al. (2014). Nature Comm 5. (PMID: 25182477)
[5] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
ATTC, American Tissue Culture Collection
DBD, DNA Binding Domain
DMSO, Dimethyl Sulfoxide
HEK, Human Embryonic Kidney
LBD, Ligand Binding Domain
MIE, Molecular Initiating Event

OT, Odyssey Thera
PCA, Protein-Fragment Complementation
PPAR, Peroxisome Proliferator-Activated Receptor
SRC-1, Steroid Receptor Coactivator 1
YFP, Yellow Fluorescent Protein

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information:

Tox21_AR_BLA_Agonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Androgen Receptor Agonist Transactivation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

Androgen receptor (AR), a nuclear hormone receptor, plays critical roles in the development and differentiation of male embryos, in the initiation and maintenance of spermatogenesis and in AR-dependent prostate cancer and other androgen related diseases. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like AR may cause disruption of normal endocrine function as well as interfere with metabolic homeostasis, reproduction, developmental and behavioral functions. To identify the compounds that inhibit AR signaling, an AR-UAS-bla GripTite™ cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under the control of an Upstream Activator Sequence (UAS) stably integrated into HEK293 cells was used to screen the Tox21 chemical library. This experimental system expresses a fusion protein of a rodent androgen receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic androgen binding compounds stimulates β-lactamase reporter gene expression.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring fluorescence emission resulting from xenobiotic AR gene expression.

Experimental System:

GeneBLAzer® AR-UAS-bla GripTite™ 293 cells contain the ligand-binding domain (LBD) of the rat androgen receptor (AR) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-bla GripTite™ 293 cell line. This portion of the rat AR is identical to the human AR in the conserved LBD and differs from the human sequence at five residues in the hinge region. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-AR (LBD) fusion protein, the protein binds to the UAS, resulting in expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM, high glucose	Invitrogen	Invitrogen/11965

Opti-MEM	Invitrogen	Invitrogen/11058
Dialyzed FBS	Invitrogen	Invitrogen/26400
HEPES	Invitrogen	Invitrogen/15630
NEAA	Invitrogen	Invitrogen/11140
Sodium pyruvate	Invitrogen	Invitrogen/11360
Penicillin and Streptomycin	Invitrogen	Invitrogen/15140
Hygromycin	Invitrogen	Invitrogen/10687
Zeocin	Invitrogen	Invitrogen/R250-01
0.05% Trypsin-EDTA	Invitrogen	Invitrogen/25300
Recovery Cell Culture Freezing Medium	Invitrogen	Invitrogen/12648
Black-clear bottom 1536 well plates	Greiner	Greiner/789092F
LiveBLAzer B/G FRET substrate	Invitrogen	Invitrogen/K1028
Multidrop COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD dispenser	Beckman Coulter	Beckman Coulter
Envision Plate Reader	Perkin Elmer	Perkin Elmer
R1881 or Methyltrienolone	Perkin Elmer	Perkin Elmer/ NLP005005MG

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM, high glucose	90%	-	90%	-
Opti-MEM	-	90%	-	-
Dialyzed FBS	10%	10%	10%	-
HEPES	25mM	-	25mM	-
NEAA	0.1mM	0.1mM	0.1mM	-
Sodium pyruvate	1mM	1mM	1mM	-
Penicillin and Streptomycin	100U/ml and 100 µg/ml	100U/ml and 100 µg/ml	100U/ml and 100 µg/ml	-
Hygromycin	80 µg/ml	-	-	-
Zeocin	80 µg/ml	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 -1ml frozen cells of AR-bla were taken in pre-warmed 10ml of thaw medium for centrifuging

1.2.2 -Thaw medium is used to re-suspend the pellet

1.2.3 -Seed the cells at 2 million per T-75 flask with thaw medium

1.3. Propagation method

1.3.1 -Detach the cells from the flask using 0.05% Trypsin
1.3.2 -The cells are re-seeded in T-225 flask at 3-4 million

2. Assay Protocol
   2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing
   2.2 -Resuspend the pellet with assay medium
   2.3 -Plate the cells in black-clear bottom 1536 well plate at 2000/well/6 µL through 8 tip Multidrop plate dispenser
   2.4 -Incubate at 37°C for 5hrs
   2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool
   2.6 -Incubate at 37°C for 16hrs
   2.7 -Add 1 µL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)
   2.8 -Incubate at room temperature for 2hrs
   2.9 -Read the fluorescence intensity through Envision plate reader

AR-bla cells were dispensed at 2,000 cells/6 µL/well of assay medium into black wall/clear-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37°C and 5% CO_2 for 4 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The plates were incubated at 37°C and 5% CO_2 for 16 h. Then 1 µL of LiveBLAzer™ B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The plates were incubated at room temperature for 2 h, and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [3]. GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang

NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 androgen receptor beta lactamase agonist assay screened a library of diverse environmental chemicals to probe for xenobiotic androgen receptor ligand-binding and potential to induce androgen-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 37 hour incubation of cells with test compounds a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for 2 hours. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known AR agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation. Concentration-response relationships were determined by monitoring luminescent signals relative to DMSO-only exposures which provided a signal baseline, and to a known androgen receptor agonist (Methyltrienolone) as a positive control, which provided an indication of 100% androgen receptor activation.

Scientific Principles:

Androgen receptors have pleiotropic regulatory roles in a diverse range of tissues; particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological

processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous androgenic signaling pathways.

The Tox21 AR bla assays are qHTS format assays which measured the ability of a chemical to interact with AR by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses rat AR in a one-hybrid GAL4 system to quantify xenobiotic androgen receptor agonism**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.198
Neutral control median absolute deviation, by plate:	6.374
Positive control well median response value, by plate:	99.356
Positive control well median absolute deviation, by plate:	21.535
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.15
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	15.51
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-412
Signal-to-background (median across all plates, using negative control wells):	NA

CV (median across all plates): -28.54

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor agonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

Beta lactamase expression in the AR_BLA_Agonist_ratio assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (Methyltrienolone; 100% activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of Methyltrienolone (R1881) activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_LUC_MDAKB2_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.303
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [4].

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Tox21_AR_BLA_Antagonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Androgen Receptor Antagonist Transactivation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

Androgen receptor (AR), a nuclear hormone receptor, plays critical roles in the development and differentiation of male embryos, in the initiation and maintenance of spermatogenesis and in AR-dependent prostate cancer and other androgen related diseases. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like AR may cause disruption of normal endocrine function as well as interfere with metabolic homeostasis, reproduction, developmental and behavioral functions. To identify the compounds that inhibit AR signaling, an AR-UAS-bla GripTite™ cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under the control of an Upstream Activator Sequence (UAS) stably integrated into HEK293 cells was used to screen the Tox21 chemical library. This experimental system expresses a fusion protein of a rodent androgen receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds stimulates β-lactamase reporter gene expression, and AR antagonism by test compounds results in decreased signal production relative to cyproterone acetate in the presence of a known AR agonist (R1881). To detect loss of signal due to compound cytotoxicity, a CellTiter-Glo fluorescence assay to measure ATP production was run concurrently in all wells using tetraoctylammonium bromide as a positive control for cell death.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring fluorescence emission resulting from xenobiotic AR gene expression.

Experimental System:

GeneBLAzer® AR-UAS-bla GripTite™ 293 cells contain the ligand-binding domain (LBD) of the rat androgen receptor (AR) fused to the DNA-binding domain of GAL4 stably integrated in the cell line. This portion of the rat AR is identical to the human AR in the conserved LBD and differs from the human sequence at five residues in the hinge region. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). This assay is performed with small amounts of a known AR agonist (R1881) added to each well, and when an antagonistic compound binds to the LBD of the GAL4-AR fusion protein binding to the UAS is inhibited, interfering with expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 (Dohr et al. 1995). The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid (Bylund et al. 2004). HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

QUALITY CONTROL PRECAUTIONS:

1. Cell culture is maintained by passaging twice a week and should not reach more than 90% confluence
2. The assay should be performed in black-clear bottom 1536 well plates, so the bottom of the plates should not be touched

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM, high glucose	Invitrogen	Invitrogen/11965
Opti-MEM	Invitrogen	Invitrogen/11058
Dialyzed FBS	Invitrogen	Invitrogen/26400
HEPES	Invitrogen	Invitrogen/15630
NEAA	Invitrogen	Invitrogen/11140
Sodium pyruvate	Invitrogen	Invitrogen/11360
Penicillin and Streptomycin	Invitrogen	Invitrogen/15140
Hygromycin	Invitrogen	Invitrogen/10687
Zeocin	Invitrogen	Invitrogen/R250-01
0.05% Trypsin-EDTA	Invitrogen	Invitrogen/25300
Recovery Cell Culture Freezing Medium	Invitrogen	Invitrogen/12648
Black-clear bottom 1536 well plates	Greiner	Greiner/789092F
LiveBLAzer B/G FRET substrate	Invitrogen	Invitrogen/K1028
CellTiter-Glo Assay Custom Solution	Promega	Promega/X2371
Multidrop COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD dispenser	Beckman Coulter	Beckman Coulter
Envision Plate Reader	Perkin Elmer	Perkin Elmer
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
Cyproterone acetate (Antagonist control compound)	Sigma Aldrich	Sigma Aldrich/C3412

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM, high glucose	90%	-	90%	-
Opti-MEM	-	90%	-	-
Dialyzed FBS	10%	10%	10%	-
HEPES	25mM	-	25mM	-
NEAA	0.1mM	0.1mM	0.1mM	-
Sodium pyruvate	1mM	1mM	1mM	-

Penicillin and Streptomycin	100U/ml and 100 µg/ml	100U/ml and 100 µg/ml	100U/ml and 100 µg/ml	-
Hygromycin	80 µg/ml	-	-	-
Zeocin	80 µg/ml	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 -1ml frozen cells of AR-bla were taken in pre-warmed 10ml of thaw medium for centrifuging

1.2.2 -Thaw medium is used to re-suspend the pellet

1.2.3 -Seed the cells at 2 million per T-75 flask with thaw medium

1.3. Propagation method

1.3.1 -Detach the cells from the flask using 0.05% Trypsin

1.3.2 -The cells are re-seeded in T-225 flask at 3-4 million

2. Assay Protocol

2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing

2.2 -Resuspend the pellet with assay medium

2.3 -Plate the cells in black-clear bottom 1536 well plate at 2000/well/6 µL through 8 tip Multidrop plate dispenser

2.4 -Incubate at 37 °C for 5hrs

2.5 -Add 1uL of assay buffer by using single tip of a plate dispenser (Bioraptr) into bottom 1/3rd part of 2 and 3 columns

2.6 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.7 -Add 1µL of 10nM (final) R1881 by using single tip of a plate dispenser (Bioraptr) into all the wells except the buffer dispensed wells of bottom 1/3rd part of 2 and 3 columns

2.8 -Incubate at 37 °C for 16hrs

2.9 -Add 1 µL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)

2.10 -Incubate at room temperature for 2hrs

2.11 -Read the fluorescence intensity through Envision plate reader

2.12 -Add 4µL of CellTiter-Glo assay reagent using a single tip of a plate dispenser (Bioraptr)

2.13 -Incubate at room temperature for 30min

2.14 -Read the luminescence through ViewLux plate reader

AR-bla cells were dispensed at 2,000 cells/5 µL of assay medium per well into black wall/clear-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37 °C and 5% CO2 for 4 hr, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1 µL of agonist (R1881) in culture medium using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The plates were incubated at a 37 °C and 5% CO2 for 16 h. After 1 µL of LiveBLAzerTM B/G FRET substrate was added using a Bioraptr FRD, the plates were incubated at room temperature for 2 h and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 4 µL/well of CellTiter-Glo reagent was added into the assay plates using a Bioraptr FRD. After 30 min incubation

at room temperature, the luminescence intensity in the plates was measured using a ViewLux (PerkinElmer) plate reader.

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) (Michael et al. 2008). GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Assay Source Contact Information:

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National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

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Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None Reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 androgen receptor beta lactamase antagonist assay screened a library of diverse environmental compounds to probe for xenobiotic androgen receptor ligand-binding and potential to suppress androgen-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 16 hour incubation of cells with test compounds a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for 2 hours. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation. Concentration-response relationships were determined by monitoring FRET signals relative to DMSO-only exposures which provided a signal baseline, and to a known androgen receptor antagonist (Cyproterone acetate) as a positive control which provided a reference for 100% androgen receptor inhibition, as assessed in the presence of 0.01 µM R1881, a known AR agonist.

Scientific Principles:

Androgen receptors have pleiotropic regulatory roles in a diverse range of tissues; particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous androgenic signaling by monitoring the decrease in luminescent signals relative to a known androgen receptor antagonist (Cyproterone acetate), an indicator of androgenic interference.

The Tox21 AR bla assays are qHTS format assays which measured the ability of a chemical to interact with AR by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses rat AR and a one-hybrid GAL4 system to quantify xenobiotic androgen receptor antagonism**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a

putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

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Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.188
Neutral control median absolute deviation, by plate:	6.931
Positive control well median response value, by plate:	-100.309
Positive control well median absolute deviation, by plate:	3.043
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.71
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-14.97
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	510.39
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-36.98

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor antagonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

Beta lactamase expression in the AR_BLA_Antagonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized to positive controls (Cyproterone acetate; 100% inhibition) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of cyproterone acetate (a known AR inhibitor) activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both

increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

Tox21_AR_LUC_MDAKB2_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.349
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.092

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [4].

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Tox21_AR_LUC_MDAKB2_Agonist

Assay Name: Tox21 Luciferase MDA-KB2 Androgen Receptor Agonist Transactivation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

Androgen receptor (AR), a nuclear hormone receptor, plays critical roles in the development and differentiation of male embryos, in the initiation and maintenance of spermatogenesis and in AR-dependent prostate cancer and other androgen related diseases. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like AR may cause disruption of normal endocrine function as well as interfere with metabolic homeostasis, reproduction, developmental and behavioral functions. To identify the compounds that activate AR signaling, MDA-kb2 AR-luc cell line (ATCC; [1]) was used to screen the Tox21 10K compound library by monitoring the increase in luminescent signals produced following androgen receptor ligand-binding and luciferase reporter gene transcription. MDA-kb2 AR-luc cell line is human breast carcinoma cell line that was stably transfected with a luciferase reporter gene under control of the MMTV promoter containing response elements for both androgen receptor (AR) and glucocorticoid receptor (GR).

1.2. Assay Definition

Assay Throughput:

Stably transfected MDA-kb2 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring luminescence resulting from AR gene expression.

Experimental System:

The MDA-kb2 AR-luc cell line was derived from epithelial breast cancer cell line, MDA-MB-453 (originally obtained in 1976 from pleural effusion of metastatic carcinoma from 48-yo Caucasian female) by stable transfection with a mouse mammary tumor virus (MMTV) neomycin-resistant luciferase reporter gene construct. MDA-MB-453 cells have fibroblastic morphology and were selected for transformation due to high levels of functional, endogenous androgen and glucocorticoid receptors [while estrogen receptor (ER) α and progesterone receptor (PR) mRNA are not detectable, and ERβ is apparently expressed at very low levels]. This cell line expresses firefly luciferase under control of a MMTV promoter that contains response elements for both GR and AR. MDA-kb2 may be used in an in vitro assay to screen androgen agonist and antagonists and to characterize its specificity and sensitivity to endocrine disrupting chemicals [1].

Xenobiotic Biotransformation Potential:

Metabolic activity has been examined for the parental MDA-MB-453 cells, and CYP1A1 and CYP1B1 have been shown to be inducible following TCDD exposures, with cells shown to respond to exposure to AhR agonists with highly preferential induction of CYP1B1 as opposed to CYP1A1 [2-5].

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
Leibovitz's L-15 Medium	ATCC	ATCC / 30-2008
Fetal Bovine Serum	Hyclone	Hyclone / SH30071.03
Penicillin/Streptomycin	Invitrogen	Invitrogen / 15140

Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen / 25300
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo(TM) Luciferase Assay System	Promega	Promega / E6120
R1881 or Methyltrienolone (Agonist control compound)	Perkin Elmer	Perkin Elmer/ NLP005005MG

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Leibovitz's L-15 Medium	100%	100%	100%	-
Fetal Bovine Serum	10%	10%	10%	-
Penicillin & Streptomycin	100U/ml & 100ug/ml	100U/ml & 100ug/ml	100U/ml & 100ug/ml	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw/culture medium and then centrifuge

1.2.2 -Resuspend the pellet with the thaw/culture medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture medium

1.3.2 -Passage cells at 6-7 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture/assay medium

2.2 -Dispense 3000 cells/5µL/well (for agonist mode) into 1536-well tissue treated white/solid bottom plates using a 8 tip dispenser (Multidrop)

2.3 -Incubate the plates for 5hrs at 37°C and 0% CO_2

2.4 -Transfer 23nL of compounds from the library collection (0.59nM to 92µM) and positive control

2.5 -Incubate the plates for 16hrs at 37°C and 0% CO_2

2.6 -Then add 5µl of ONE-Glo™ Luciferase reagent using a single tip dispenser (Bioraptr)

2.7 -Incubate the plates at room temperature for 30min

2.8 -Measure luminescence by ViewLux plate reader

MDA-kb2 AR-luc cells were dispensed at 3,000 cells/5µL/well of culture medium into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 °C and 0% CO_2 for 5h, 23nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The plates were incubated at 37 °C and 0% CO_2 for 16h. Then 5µL of ONE-Glo reagent (Promega, Madison, WI) was added to each plate using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA) and luminescence was quantified on a ViewLux (PerkinElmer, Shelton, CT) plate reader after 30 min incubation at room temperature.

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [6].

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 androgen receptor luciferase agonist assay screened a large library of diverse environmental compounds to probe for xenobiotic androgenic activity and potential to induce

androgen-dependent transcription, monitored through luciferase reporter gene signal activation using an AR-luciferase reporter gene construct. The assay is run in triplicate on a 1536-well microplate and bioluminescence was measured following 16 hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase ATP detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Androgen receptors have pleiotropic regulatory roles in a diverse range of tissues; particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous androgenic signaling by monitoring the increase in luminescent signals relative to a known androgen receptor agonist (Methyltrienolone) as a positive control, and indicator of androgenic activity.

The Tox21 MDA-kb2 AR luciferase assays are qHTS format assays which measured the ability of a chemical to interact with AR by monitoring modulation of luminescent reporter gene signals. This assay utilized an epithelial breast cancer cell line which expresses firefly luciferase under control of a MMTV promoter that contains androgen response elements to quantify xenobiotic androgen receptor agonism**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Wilson, V. S., Bobseine, K., Lambright, C. R., & Gray, L. (2002). "A novel cell line, MDA-kb2, that stably expresses an androgen-and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists". Toxicol Sci 66(1), 69-81. (PMID: 11861974)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.111
Neutral control median absolute deviation, by plate:	3.344
Positive control well median response value, by plate:	99.51
Positive control well median absolute deviation, by plate:	7.91
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.66
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	29.73
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-893.63
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-29.54

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor agonism and gene expression, as measured by monitoring luminescent production by luciferase reporter gene under control of androgen response element promoters.

Analytical Elements:

The Tox21 MDA-KB2AR-luciferase agonist assay was monitored for increased luminescence (gain-of-signal) relative to methyltrienolone (positive control) signal, using DMSO (negative control) as a baseline for chemical-AR activity, and response was reported as a percent of positive control activity. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.010
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Wilson, V. S., et al. (2002). Toxicol Sci 66(1): 69-81. (PMID: 11861974)
[2] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[3] Spink, D. C., et al. (1998). Carcinogenesis 19(2): 291-298. (PMID: 9498279)
[4] Angus, W. G., et al. (1999). Carcinogenesis 20(6): 947-955. (PMID: 10357772)
[5] Spink, B., et al. (2002). Toxicol In Vitro 16(6): 695-704. (PMID: 12423652)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

Author of Revisions:

5. Supporting Information

Tox21_AR_LUC_MDAKB2_Agonist_3uM_Nilutamide

Assay Name: Tox21 Luciferase MDA-KB2 Androgen Receptor Agonist Transactivation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

Androgen receptor (AR), a nuclear hormone receptor, plays critical roles in the development and differentiation of male embryos, in the initiation and maintenance of spermatogenesis and in AR-dependent prostate cancer and other androgen related diseases. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like AR may cause disruption of normal endocrine function as well as interfere with metabolic homeostasis, reproduction, developmental and behavioral functions. To identify the compounds that activate AR signaling, MDA-kb2 AR-luc cell line (ATCC; [1]) was used to screen the Tox21 10K compound library by monitoring the increase in luminescent signals produced following androgen receptor ligand-binding and luciferase reporter gene transcription. MDA-kb2 AR-luc cell line is human breast carcinoma cell line that was stably transfected with a luciferase reporter gene under control of the MMTV promoter containing response elements for both androgen receptor (AR) and glucocorticoid receptor (GR).

1.2. Assay Definition

Assay Throughput:

Stably transfected MDA-kb2 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring luminescence resulting from AR gene expression.

Experimental System:

The MDA-kb2 AR-luc cell line was derived from epithelial breast cancer cell line, MDA-MB-453 (originally obtained in 1976 from pleural effusion of metastatic carcinoma from 48-yo Caucasian female) by stable transfection with a mouse mammary tumor virus (MMTV) neomycin-resistant luciferase reporter gene construct. MDA-MB-453 cells have fibroblastic morphology and were selected for transformation due to high levels of functional, endogenous androgen and glucocorticoid receptors [while estrogen receptor (ER) α and progesterone receptor (PR) mRNA are not detectable, and ERβ is apparently expressed at very low levels]. This cell line expresses firefly luciferase under control of a MMTV promoter that contains response elements for both GR and AR. MDA-kb2 may be used in an in vitro assay to screen androgen agonist and antagonists and to characterize its specificity and sensitivity to endocrine disrupting chemicals [1].

Xenobiotic Biotransformation Potential:

Metabolic activity has been examined for the parental MDA-MB-453 cells, and CYP1A1 and CYP1B1 have been shown to be inducible following TCDD exposures, with cells shown to respond to exposure to AhR agonists with highly preferential induction of CYP1B1 as opposed to CYP1A1 [2-5].

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
Leibovitz's L-15 Medium	ATCC	ATCC / 30-2008

Fetal Bovine Serum	Hyclone	Hyclone / SH30071.03
Penicillin/Streptomycin	Invitrogen	Invitrogen / 15140
Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen / 25300
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo(TM) Luciferase Assay System	Promega	Promega / E6120
R1881 or Methyltrienolone (Agonist control compound)	Perkin Elmer	Perkin Elmer/ NLP005005MG
Nilutamide	Sigma-Aldrich	Sigma-Aldrich N8534

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Leibovitz's L-15 Medium	100%	100%	100%	-
Fetal Bovine Serum	10%	10%	10%	-
Penicillin & Streptomycin	100U/ml & 100ug/ml	100U/ml & 100ug/ml	100U/ml & 100ug/ml	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw/culture medium and then centrifuge

1.2.2 -Resuspend the pellet with the thaw/culture medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture medium

1.3.2 -Passage cells at 6-7 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture/assay medium

2.2 -Dispense 3000 cells/4µL/well (for agonist mode) into 1536-well tissue treated white/solid bottom plates using a 8 tip dispenser (Multidrop)

2.3 -Incubate the plates for 5hrs at 37°C and 0% CO_2

2.4 - Add 1 µL of 15.0 µM Nilutamide/assay medium (3 µM final concentration) was added using a dispenser.

2.4 -Transfer 23nL of compounds from the library collection (0.59nM to 92µM final assay concentrations) and positive control
2.5 -Incubate the plates for 16hrs at 37°C and 0% CO_2
2.6 -Then add 5µl of ONE-Glo™ Luciferase reagent using a single tip dispenser (Bioraptr)
2.7 -Incubate the plates at room temperature for 30min
2.8 -Measure luminescence by ViewLux plate reader

MDA-kb2 AR-luc cells were dispensed at 3,000 cells/4µL/well of culture medium into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 °C and 0% CO_2 for 5h, 1 µL of 15.0 µM Nilutamide/assay medium was added using a dispenser followed by the compound transfer. 23nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The plates were incubated at 37 °C and 0% CO_2 for 16h. Then 5µL of ONE-Glo reagent (Promega, Madison, WI) was added to each plate using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA) and luminescence was quantified on a ViewLux (PerkinElmer, Shelton, CT) plate reader after 30 min incubation at room temperature.

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [6].

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated gene expression and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity

prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 androgen receptor luciferase agonist assay screened a large library of diverse environmental compounds to probe for xenobiotic androgenic activity and potential to induce androgen-dependent transcription, monitored through luciferase reporter gene signal activation using an AR-luciferase reporter gene construct. The assay is run in triplicate on a 1536-well microplate and bioluminescence was measured following 16 hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase ATP detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Androgen receptors have pleiotropic regulatory roles in a diverse range of tissues; particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous androgenic signaling by monitoring the increase in luminescent signals relative to a known androgen receptor agonist (Methyltrienolone) as a positive control, and indicator of androgenic activity.

The Tox21 MDA-kb2 AR luciferase assays are qHTS format assays which measured the ability of a chemical to interact with AR by monitoring modulation of luminescent reporter gene signals. This assay utilized an epithelial breast cancer cell line which expresses firefly luciferase under control of a MMTV promoter that contains androgen response elements to quantify xenobiotic androgen receptor agonism**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the

MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Wilson, V. S., Bobseine, K., Lambright, C. R., & Gray, L. (2002). "A novel cell line, MDA-kb2, that stably expresses an androgen-and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists". Toxicol Sci 66(1), 69-81. (PMID: 11861974)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	1.191
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	7.51
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.74
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	83.93
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor agonism and gene expression, as measured by monitoring luminescent production by luciferase reporter gene under control of androgen response element promoters.

Analytical Elements:

This assay is a counter-screen that is run in agonist mode but with the inclusion of an AR antagonist, nilutamide, to block agonist activity. Results should be compared to TOX21_AR_LUC_MDAKB2_Agonist (AEID 764) which was run in the absence of nilutamide. Chemicals with activity in AEID 764 but not in AEID 1822 may be interpreted as competitive AR agonists. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of

the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

TOX21_AR_LUC_MDAKB2_Agonist
ATG_AR_TRANS_up
NVS_NR_cAR
NVS_NR_hAR
NVS_NR_rAR
OT_AR_ARELUC_AG_1440
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
Tox21_AR_BLA_Agonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.768
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Wilson, V. S., et al. (2002). Toxicol Sci 66(1): 69-81. (PMID: 11861974)
[2] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[3] Spink, D. C., et al. (1998). Carcinogenesis 19(2): 291-298. (PMID: 9498279)
[4] Angus, W. G., et al. (1999). Carcinogenesis 20(6): 947-955. (PMID: 10357772)
[5] Spink, B., et al. (2002). Toxicol In Vitro 16(6): 695-704. (PMID: 12423652)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

Author of Revisions:

5. Supporting Information

Tox21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881

Assay Name: Tox21 Luciferase MDA-KB2 Androgen Receptor Antagonist Transactivation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

Androgen receptor (AR), a nuclear hormone receptor, plays critical roles in the development and differentiation of male embryos, in the initiation and maintenance of spermatogenesis and in AR-dependent prostate cancer and other androgen related diseases. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like AR may cause disruption of normal endocrine function as well as interfere with metabolic homeostasis, reproduction, developmental and behavioral functions. To identify the compounds that inhibit AR signaling, MDA-kb2 AR-luc cell line (ATCC; (Wilson et al. 2002)) was used to screen the Tox21 compound library using luciferase reporter gene construct downstream of endogenous androgen receptor and exposing the cells to test compounds and a small amount of AR agonist. The cytotoxicity of the Tox21 compounds against the MDA-kb2 AR-luc cell line was tested in parallel by measuring the cell viability using CellTiter-Fluor assay (see Assay Definition) in the same wells. The antagonist assay was also run with a higher concentration of agonist (AEID765) as a specificity control in which competitive antagonists should show a right-shift in potency relative to the assay with lower agonist concentration.

1.2. Assay Definition

Assay Throughput:

Stably transfected MDA-kb2 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring luminescence resulting from AR gene expression.

Experimental System:

The MDA-kb2 AR-luc cell line was derived from epithelial breast cancer cell line, MDA-MB-453 (originally obtained in 1976 from pleural effusion of metastatic carcinoma from 48-yo Caucasian female) by stable transfection with a mouse mammary tumor virus (MMTV) neomycin-resistant luciferase reporter gene construct. MDA-MB-453 cells have fibroblastic morphology and were selected for transformation due to high levels of functional, endogenous androgen and glucocorticoid receptors (while estrogen receptor (ER) α and progesterone receptor (PR) mRNA are not detectable, and ERβ is apparently expressed at very low levels). This cell line expresses firefly luciferase under control of a MMTV promoter that contains response elements for both GR and AR. MDA-kb2 may be used in an in vitro assay to screen androgen agonist and antagonists and to characterize its specificity and sensitivity to endocrine disrupting chemicals (Wilson et al. 2002).

Xenobiotic Biotransformation Potential:

Metabolic activity has been examined for the parental MDA-MB-453 cells, and CYP1A1 and CYP1B1 have been shown to be inducible following TCDD exposures, with cells shown to respond to exposure to AhR agonists with highly preferential induction of CYP1B1 as opposed to CYP1A1 (Angus et al. 1999, Dohr et al. 1995, Spink BC et al. 2002, Spink David C et al. 1998).

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
Leibovitz's L-15 Medium	ATCC	ATCC / 30-2008
Fetal Bovine Serum	Hyclone	Hyclone / SH30071.03
Penicillin/Streptomycin	Invitrogen	Invitrogen / 15140
Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen / 25300
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo(TM) Luciferase Assay System	Promega	Promega / E6120
CellTiter-Fluor (TM) Cell Viability Assay	Promega	Promega / G6082

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Leibovitz's L-15 Medium	100%	100%	100%	-
Fetal Bovine Serum	10%	10%	10%	-
Penicillin & Streptomycin	100U/ml & 100ug/ml	100U/ml & 100ug/ml	100U/ml & 100ug/ml	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw/culture medium and then centrifuge

1.2.2 -Resuspend the pellet with the thaw/culture medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture medium

1.3.2 -Passage cells at 6-7 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture/assay medium

2.2 -Dispense 3000 cells/4 µL/well into 1536-well tissue treated white/solid bottom plates using an 8 tip dispenser (Multidrop)

2.3 - Incubate the plates for 5 hrs at 37C and 0% CO_2

2.4 - Transfer 23nL of compounds from the library collection (0.59nM to 92µM) and positive control
2.5 - Add 1 µL/well of 2.55 nM R1881agonist (final concentration of 0.5 nM,) in culture medium using Bioraptr Flying Reagent Dispenser workstation (Beckman Coulter, Indianapolis, IN, USA).
2.6 -Incubate the plates for 15.5hr at 37°C and 0% CO_2
2.7 -Add 1µl of CellTiter-Fluor™ Cell Viability Assay reagent using a single tip dispenser (Bioraptr)
2.8 -Incubate the plates at room temperature or 37°C for 30min
2.9 -Measure fluorescence by ViewLux plate reader
2.10 -Then add 4µl of ONE-Glo™ Luciferase reagent using a single tip dispenser (Bioraptr)
2.11 -Incubate the plates at room temperature for 30min
2.12 -Measure luminescence by ViewLux plate reader

MDA-kb2 cells were dispensed at 3,000 cells/4 µL/well of culture medium into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37°C and 0% CO_2 for 5 hr, 23nL of compounds dissolved in DMSO, positive controls or DMSO-only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition 1 µL of agonist (2.5 nM R1881) in culture medium using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The plates were incubated at a 37°C and 0% CO_2 for 16 h. After 15.5 h incubation, 1µL of CellTiter-Fluor reagent (Promega, Madison, WI) was added using a Bioraptr FRD to each plate for measuring cytotoxicity. The fluorescence intensity was quantified on a ViewLux plate reader (PerkinElmer, Shelton, CT) following 30 min incubation at 37°C and 0% CO_2. For measuring luciferase reporter gene activity, 4 µL of ONE-Glo reagent (Promega) was added to each plate using a Bioraptr FRD and luminescence was quantified on a ViewLux (PerkinElmer) plate reader after 30 min incubation at room temperature.

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) (Michael et al. 2008).

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 androgen receptor luciferase antagonist assay screened a large library of diverse environmental compounds to probe for xenobiotic androgenic activity and potential to inhibit androgen-dependent transcription, monitored through luciferase reporter gene signal activity using an AR-luciferase reporter gene construct. The assay is run in triplicate on a 1536-well microplate and bioluminescence was measured following 15.5 hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in antagonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. To help distinguish true antagonistic activity from cytotoxic effects, this assay was multiplexed with a fluorescence-based cell viability assay which measured conserved and constitutive protease activity within live cells (Promega). Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation. Concentration-response relationships were determined by monitoring luminescent signals relative to DMSO-only exposures which provided a signal baseline, and to a known androgen receptor antagonist (Nilutamide) as a positive control which provided a reference for 100% androgen receptor inhibition, as assessed in the presence of 0.5 nM R1881, a known AR agonist.

Scientific Principles:

Androgen receptors have pleiotropic regulatory roles in a diverse range of tissues; particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental chemicals can result in hormonal cancers, impaired reproductive development and infertility. This assay is designed to screen a large, structurally diverse chemical library to identify compounds

capable of interference with endogenous androgenic signaling by monitoring the relative decrease in production of luminescent signals using a known androgen receptor antagonist (nilutamide) as a positive control, and indicator of AR inhibition.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Wilson, V. S., Bobseine, K., Lambright, C. R., & Gray, L. (2002). "A novel cell line, MDA-kb2, that stably expresses an androgen-and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists". Toxicol Sci 66(1), 69-81. (PMID: 11861974)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.68
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.896
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.68
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-11.52
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor antagonism and inhibited gene expression, as measured by monitoring production of luminescence resulting from luciferase reporter gene under the regulation androgen response element promoters. This assay was also run with 10 nM R1881 (AEID 765) which can be used to distinguish competitive antagonists from non-competitive or cytotoxic chemicals.

Analytical Elements:

The Tox21 MDA-KB2AR-luciferase agonist assay was monitored for decreased activity (loss-of-signal) relative to DMSO (negative control) and nilutamide signal (positive control, 100% antagonist activity) measured in the presence of small amounts (0.5 nM) of a known agonist (R1881). Relationships are modeled. Concentration-response relationships were determined based on a

range of 15 chemical concentrations using DMSO as a signal baseline, and response was reported as a percent of positive control activity (100% inhibition). All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

Tox21_AR_BLA_Antagonist_ratio

Tox21_AR_LUC_MDAKB2_Antagonist_10nM_R1881

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.35
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.13

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and

ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Wilson, V. S., et al. (2002). Toxicol Sci 66(1): 69-81. (PMID: 11861974)
[2] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[3] Spink, D. C., et al. (1998). Carcinogenesis 19(2): 291-298. (PMID: 9498279)
[4] Angus, W. G., et al. (1999). Carcinogenesis 20(6): 947-955. (PMID: 10357772)
[5] Spink, B., et al. (2002). Toxicol In Vitro 16(6): 695-704. (PMID: 12423652)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

Tox21_AR_LUC_MDAKB2_Antagonist_10nM_R1881

Assay Name: Tox21 Luciferase MDA-KB2 Androgen Receptor Antagonist Transactivation Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

Androgen receptor (AR), a nuclear hormone receptor, plays critical roles in the development and differentiation of male embryos, in the initiation and maintenance of spermatogenesis and in AR-dependent prostate cancer and other androgen related diseases. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like AR may cause disruption of normal endocrine function as well as interfere with metabolic homeostasis, reproduction, developmental and behavioral functions. To identify the compounds that inhibit AR signaling, MDA-kb2 AR-luc cell line (ATCC; (Wilson et al. 2002)) was used to screen the Tox21 compound library using luciferase reporter gene construct downstream of endogenous androgen receptor and exposing the cells to test compounds and a small amount of AR agonist. The cytotoxicity of the Tox21 compounds against the MDA-kb2 AR-luc cell line was tested in parallel by measuring the cell viability using CellTiter-Fluor assay (see Assay Definition) in the same wells. This assay serves as a specificity control for AEID 1816 through use of a higher agonist concentration which should result in a right shift in potency for competitive AR antagonists relative to the lower agonist concentration used in AEID 1816.

1.2. Assay Definition

Assay Throughput:

Stably transfected MDA-kb2 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring luminescence resulting from AR gene expression.

Experimental System:

The MDA-kb2 AR-luc cell line was derived from epithelial breast cancer cell line, MDA-MB-453 (originally obtained in 1976 from pleural effusion of metastatic carcinoma from 48-yo Caucasian female) by stable transfection with a mouse mammary tumor virus (MMTV) neomycin-resistant luciferase reporter gene construct. MDA-MB-453 cells have fibroblastic morphology and were selected for transformation due to high levels of functional, endogenous androgen and glucocorticoid receptors (while estrogen receptor (ER) α and progesterone receptor (PR) mRNA are not detectable, and ERβ is apparently expressed at very low levels). This cell line expresses firefly luciferase under control of a MMTV promoter that contains response elements for both GR and AR. MDA-kb2 may be used in an in vitro assay to screen androgen agonist and antagonists and to characterize its specificity and sensitivity to endocrine disrupting chemicals (Wilson et al. 2002).

Xenobiotic Biotransformation Potential:

Metabolic activity has been examined for the parental MDA-MB-453 cells, and CYP1A1 and CYP1B1 have been shown to be inducible following TCDD exposures, with cells shown to respond to exposure to AhR agonists with highly preferential induction of CYP1B1 as opposed to CYP1A1 (Angus et al. 1999, Dohr et al. 1995, Spink BC et al. 2002, Spink David C et al. 1998).

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
Leibovitz's L-15 Medium	ATCC	ATCC / 30-2008
Fetal Bovine Serum	Hyclone	Hyclone / SH30071.03
Penicillin/Streptomycin	Invitrogen	Invitrogen / 15140
Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen / 25300
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo(TM) Luciferase Assay System	Promega	Promega / E6120
CellTiter-Fluor (TM) Cell Viability Assay	Promega	Promega / G6082

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Leibovitz's L-15 Medium	100%	100%	100%	-
Fetal Bovine Serum	10%	10%	10%	-
Penicillin & Streptomycin	100U/ml & 100ug/ml	100U/ml & 100ug/ml	100U/ml & 100ug/ml	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw/culture medium and then centrifuge

1.2.2 -Resuspend the pellet with the thaw/culture medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture medium

1.3.2 -Passage cells at 6-7 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture/assay medium

2.2 -Dispense 3000 cells/4 µL/well into 1536-well tissue treated white/solid bottom plates using an 8 tip dispenser (Multidrop)

2.3 - Incubate the plates for 5 hrs at 37C and 0% CO_2

2.4 - Transfer 23nL of compounds from the library collection (0.59nM to 92µM) and positive control
2.5 - Add 1 µL/well of 50 nM R1881 agonist (final concentration 10 nM) in culture medium using Bioraptr Flying Reagent Dispenser workstation (Beckman Coulter, Indianapolis, IN, USA).
2.6 -Incubate the plates for 15.5hr at 37°C and 0% CO_2
2.7 -Add 1µl of CellTiter-Fluor™ Cell Viability Assay reagent using a single tip dispenser (Bioraptr)
2.8 -Incubate the plates at room temperature or 37°C for 30min
2.9 -Measure fluorescence by ViewLux plate reader
2.10 -Then add 4µl of ONE-Glo™ Luciferase reagent using a single tip dispenser (Bioraptr)
2.11 -Incubate the plates at room temperature for 30min
2.12 -Measure luminescence by ViewLux plate reader

MDA-kb2 cells were dispensed at 3,000 cells/4 µL/well of culture medium into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37°C and 0% CO_2 for 5 hr, 23nL of compounds dissolved in DMSO, positive controls or DMSO-only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1 µL of agonist (50 nM R1881) in culture medium using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The plates were incubated at a 37°C and 0% CO_2 for 16 h. After 15.5 h incubation, 1µL of CellTiter-Fluor reagent (Promega, Madison, WI) was added using a Bioraptr FRD to each plate for measuring cytotoxicity. The fluorescence intensity was quantified on a ViewLux plate reader (PerkinElmer, Shelton, CT) following 30 min incubation at 37°C and 0% CO_2. For measuring luciferase reporter gene activity, 4 µL of ONE-Glo reagent (Promega) was added to each plate using a Bioraptr FRD and luminescence was quantified on a ViewLux (PerkinElmer) plate reader after 30 min incubation at room temperature.

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) (Michael et al. 2008).

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote androgen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 androgen receptor luciferase antagonist assay screened a large library of diverse environmental compounds to probe for xenobiotic androgenic activity and potential to inhibit androgen-dependent transcription, monitored through luciferase reporter gene signal activity using an AR-luciferase reporter gene construct. The assay is run in triplicate on a 1536-well microplate and bioluminescence was measured following 15.5 hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in antagonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. To help distinguish true antagonistic activity from cytotoxic effects, this assay was multiplexed with a fluorescence-based cell viability assay which measured conserved and constitutive protease activity within live cells (Promega). Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation. Concentration-response relationships were determined by monitoring luminescent signals relative to DMSO-only exposures which provided a signal baseline, and to a known androgen receptor antagonist (Nilutamide) as a positive control which provided a reference for 100% androgen receptor inhibition, as assessed in the presence of 0.01 µM R1881, a known AR agonist. The assay serves as a specificity control for AEID 1816 as competitive antagonists for the androgen receptor should show a right shift in potency in this assay compared to AEID 1816 due to the use of higher concentration of agonist stimulation.

Scientific Principles:

Androgen receptors have pleiotropic regulatory roles in a diverse range of tissues; particularly in mediating the activity of endogenous androgens in the hypothalamus, pituitary, liver, prostate, and testicular tissues. Endogenous androgens are important for target gene expression in physiological processes like developmental differentiation of the male embryo, initiation and maintenance of spermatogenesis and in neuroendocrine system functioning. Endogenous androgens also influence male pubertal maturation and maintenance of secondary sexual characteristics in adults. Disruption of androgen signaling following exposure to endocrine-mimicking environmental

chemicals can result in hormonal cancers, impaired reproductive development and infertility. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous androgenic signaling by monitoring the relative decrease in production of luminescent signals using a known androgen receptor antagonist (nilutamide) as a positive control, and indicator of AR inhibition.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with androgen receptor mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that androgen receptor agonism is a molecular initiating event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in fish populations (EAGMST Approved AOP), and there is some evidence that androgen receptor activation is the MIE for a putative pathway leading to hepatocellular adenomas and carcinomas (in mouse and rat models) (AOP currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive *in vivo* studies to further investigate the involvement of AR agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Wilson, V. S., Bobseine, K., Lambright, C. R., & Gray, L. (2002). "A novel cell line, MDA-kb2, that stably expresses an androgen-and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists". Toxicol Sci 66(1), 69-81. (PMID: 11861974)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.247
Neutral control median absolute deviation, by plate:	8.706
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	2.403
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.66
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-11.43
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	403.07
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-38.35

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Androgen receptor antagonism and inhibited gene expression, as measured by monitoring production of luminescence resulting from luciferase reporter gene under the regulation androgen response element promoters. Use of the 10 nM R1881 should result in a right-shifting of AC50s for competitive AR antagonists relative to the results of the TOX21_AR_LUC_MDAKB2_Antagonist2 assay, which used 0.5 nM R1881. This is useful for distinguishing competitive antagonists from non-competitive or cytotoxic compounds.

Analytical Elements:

The Tox21 MDA-KB2AR-luciferase antagonist assay was monitored for decreased activity (loss-of-signal) relative to DMSO (negative control) and nilutamide signal (positive control, 100% antagonist activity) measured in the presence of a known agonist (R1881, 10 nM). This assay was run as a counter-screen to TOX21_AR_LUC_MDAKB2_Antagonist_0.5 nM_R1881 (AEID 1816) Results should be compared and competitive antagonists should show a right-shift in potency in AEID 765 relative to AEID 1816. Relationships are modeled. Concentration-response relationships were determined based on a range of 15 chemical concentrations using DMSO (negative control) as a signal baseline, and response was reported as a percent of positive control activity (100% inhibition). All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Androgen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

TOX21_AR_BLA_Antagonist_ratio

TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.981
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical

constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Wilson, V. S., et al. (2002). Toxicol Sci 66(1): 69-81. (PMID: 11861974)
[2] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[3] Spink, D. C., et al. (1998). Carcinogenesis 19(2): 291-298. (PMID: 9498279)
[4] Angus, W. G., et al. (1999). Carcinogenesis 20(6): 947-955. (PMID: 10357772)
[5] Spink, B., et al. (2002). Toxicol In Vitro 16(6): 695-704. (PMID: 12423652)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
AR, Androgen Receptor
ATCC, American Tissue Culture Collection
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 July 2016

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:
Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation
Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;
Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

Tox21_ERa_BLA_Agonist_ratio

Assay Name: Tox21 Beta-Lactamase-HEK293T Cell-Based qHTS Assay to Identify Small Molecule Agonists of the Estrogen Receptor Alpha (ER-alpha) Signaling Pathway

1. Assay Descriptions

1.1. Overview

Assay Summary:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify the compounds that inhibit ER signaling, an ER-alpha-UAS-bla GripTite™ cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under the control of an Upstream Activator Sequence (UAS) responsive to a GAL4-ER fusion protein stably integrated into HEK293 cells was used to screen the Tox21 chemical library. This experimental system constitutively co-expresses the fusion protein, thel human estrogen receptor alpha (ERα) ligand-binding domain coupled to GAL4 DNA-binding domain which, when activated by xenoestrogenic compounds, stimulates β-lactamase reporter gene expression.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates, incubated with test compounds for 16 hours, and reporter gene substrate added prior to monitoring fluorescence emission resulting from substrate cleavage by ERα-stimulated reporter gene expression.

Experimental System:

GeneBLAzer® ER alpha-UAS-bla GripTite™ cells contain the ligand-binding domain of the human estrogen receptor alpha (ERα) fused to the DNA-binding domain of GAL4 stably integrated into the cell line, commonly called a mammalian one-hybrid assay. These cells stably express a β-lactamase reporter gene (*bla*) under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4-ERα fusion protein, the protein binds to the UAS, resulting in expression of β-lactamase. The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
Phenol red-free DMEM	Invitrogen	Invitrogen/21063
DMEM	Invitrogen	Invitrogen/11965

Dialyzed FBS	Invitrogen	Invitrogen/26400
Charcoal stripped FBS	Invitrogen	Invitrogen/12676
NEAA	Invitrogen	Invitrogen/11140
Sodium pyruvate	Invitrogen	Invitrogen/11360
Penicillin and Streptomycin	Invitrogen	Invitrogen/15140
Hygromycin B	Invitrogen	Invitrogen/10687
Zeocin	Invitrogen	Invitrogen/R25001
Black-clear bottom 1536 well plates	Greiner	Greiner/789092F
LiveBLAzer B/G FRET substrate	Invitrogen	Invitrogen/K1028
Recovery Cell Culture Freezing Medium	Invitrogen	Invitrogen/12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen/25300
Envision Plate Reader	Perkin Elmer	Perkin Elmer
BioRAPTR FRD dispenser	Beckman Coulter	Beckman Coulter
Multidrop COMBI	Thermo Electron Corporation	Thermo Electron Corporation
Beta-Estradiol (Agonist control compound)	Sigma	Sigma/E8875

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Phenol red-free DMEM	-	98%	-	-
DMEM	90%	-	90%	-
Dialyzed FBS	10%	-	10%	-
Charcoal stripped FBS	-	2%	-	-
NEAA	0.1mM	0.1mM	0.1mM	-
Sodium pyruvate	1mM	1mM	1mM	-
Penicillin and Streptomycin	100U/ml-100µg/ml	100U/ml-100µg/ml	100U/ml-100µg/ml	-
Hygromycin B	80µg/ml	-	-	-
Zeocin	100µg/ml	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 1ml frozen cells of ERalpha-bla were taken in pre-warmed 10ml of thaw medium for centrifuging.

1.2.2 2-3ml of the thaw medium is taken to resuspend the pellet
1.2.3 The cells were seeded in T-75 flask at 2 million cells
1.3. Propagation method
1.3.1 The cells are detached using 0.05% Trypsin
1.3.2 Cells are further passaged at a density of 4-5 million cells per T-225 flask

2. Assay Protocol
2.1 Rinse the cells twice with DPBS and detach them using 0.05% Trypsin and centrifuge
2.2 Resuspend the pellet with assay buffer
2.3 Plate the cells in black-clear bottom 1536 well plate at 5000/well/5µL through 8 tip plate dispenser (Multi drop)
2.4 Incubate at 37°C for 5hrs
2.5 Transfer 23nL of the compounds from the library collection and positive control through Pintool
2.6 Incubate at 37°C for 18hrs
2.7 Add 1µL of CCF4 dye using a single tip of a plate dispenser (Bioraptr)
2.8 Incubate at room temperature for 2hrs
2.9 Read the fluorescence intensity through Envision plate reader

ER-alpha-bla cells were cultured in assay medium containing 2% charcoal stripped FBS overnight in the culture flasks before the assay was performed. The cells were dispensed at 5,000 cells/6 uL of assay medium per well into black wall/clear-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37 °C and 5% CO2 for 4 hr, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The plates were incubated at 37C and 5% CO2 for 18 hr. Then 1 uL of LiveBLAzer™ B/G FRET substrate was added using a Flying Reagent Dispenser (Aurora Discovery, San Diego, CA). After the plates were incubated at room temperature for 2 hours, fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, etc.) and signal recording (plate readout) [3]. GeneBLAzer® System is commercially available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]

U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Sakamuru, S., Martin, M. T., Reif, D. M., Judson, R. S., Houck, K. A., Casey, W., Hsieh, J. H., Shockley, K. R., Ceger, P., Fostel, J., Witt, K. L., Tong, W., Rotroff, D. M., Zhao, T., Shinn, P., Simeonov, A., Dix, D. J., Austin, C. P., Kavlock, R. J., Tice, R. R., & Xia, M. (2014). "Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway". Scientific Reports 4, 5664. (PMID: 25012808)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 estrogen receptor-β β-lactamase agonist assay screened a library of diverse environmental compounds to probe for xenoestrogenic ligand-binding and potential to induce estrogen-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. Following 18 hour incubation of cells with test compounds, a cell-permeable, FRET-based substrate, CCF4-AM, is introduced and incubated for 2 hours. Once in the cell, cytoplasmic esterases hydrolyze and trap the negatively charged CCF4 substrate in the cytosol where it can be cleaved by the *bla* reporter gene product. Activity is quantified by measuring the ratio of blue (product) to green (substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader and CellTiter-Glo assay reagent (Promega) is also incubated with test system for 30 minutes before readout to detect cell viability.

The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-

fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts.

Scientific Principles:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER agonists, GeneBLAzer® ERα-UAS-bla GripTite™ cell line (Invitrogen) has been used to screen the Tox21 library of diverse environmental compounds. ERα-UAS-bla cell line expresses a partial ERα one-hybrid GAL4 system and is stably transfected with a β-lactamase reporter gene.

The Tox21 ERα bla assays are qHTS format assays which measured the ability of a chemical to interact with estrogen receptor alpha (ERα) by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses a partial ERα and a one-hybrid GAL4 system to quantify xenoestrogenic agonism**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci U S A 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.04
Neutral control median absolute deviation, by plate:	1.12
Positive control well median response value, by plate:	99.18
Positive control well median absolute deviation, by plate:	20.81
Z' (median across all plates, using positive control wells):	0.33
SSMD (median across all plates, using positive control wells):	5
Signal-to-noise (median across all plates, using positive control wells):	87.86
Signal-to-background (median across all plates, using positive control wells):	-2485.43
CV (median across all plates):	-27.68

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Xenoestrogenic ligand-binding and ERα agonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the ERa_BLA_Agonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (17 beta-estradiol; 100% activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of 17 beta-estradiol activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Antagonist_ratio

Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 15
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.001 µM
Standard maximum concentration tested: 90 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.73
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	Yes
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	Yes
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	Yes
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	No
80-05-7	Bisphenol A	Weak	Active	Yes

1478-61-1	Bisphenol AF	NA	Active	No
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	Yes
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	NA	No
84-61-7	Dicyclohexyl phthalate	NA	Inactive	No
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	No
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	Yes
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	No
51630-58-1	Fenvalerate	NA	Inactive	Yes
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	Yes
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	No
789-02-6	o,p'-DDT	Weak	Active	No
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	Yes
57-30-7	Phenobarbital sodium	Inactive	NA	Yes
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	16	13
Inactive	4	4

In Vivo Activity	ToxCast Active	ToxCast Inactive
Active	17	13
Inactive	6	5

In Vitro Sensitivity = 55.2%
In Vitro Specificity = 50.0%
Balanced Accuracy = 52.6%

In Vivo Sensitivity = 56.7%
In Vivo Specificity = 45.5%
Balanced Accuracy = 51.1%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [4].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods

NR, Nuclear Receptors
qHTS, quantitative high-throughput screening
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

7 June 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Assay Endpoint ID: 786

Tox21_ERa_BLA_Antagonist_ratio

Assay Name: Tox21 Beta-Lactamase-HEK293T Cell-Based qHTS Assay to Identify Small Molecule Antagonists of the Estrogen Receptor Alpha (ER-alpha) Signaling Pathway

1. Assay Descriptions

1.1. Overview

Assay Summary:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify the compounds that inhibit ER signaling, an ER-alpha-UAS-bla GripTite™ cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under the control of an Upstream Activator Sequence stably integrated into HEK293 cells was used to screen the Tox21 chemical library. This experimental system constitutively co-expresses a fusion protein of a partial human estrogen receptor alpha (ERα) ligand-binding domain coupled to GAL4 DNA-binding domain which when inhibited by xenoestrogenic compounds suppresses β-lactamase reporter gene expression. To detect loss of signal due to compound cytotoxicity, a CellTiter-Glo fluorescence assay to quantify cellular ATP content was run concurrently in all wells using tetraoctylammonium bromide as a positive control for cell death.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 16 hours prior to monitoring fluorescence emission resulting from xenobiotic ERα gene expression.

Experimental System:

GeneBLAzer® ER alpha-UAS-bla GripTite™ cells contain the ligand-binding domain of the human estrogen receptor alpha (ERα) fused to the DNA-binding domain of GAL4 stably integrated into the cell line. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). This assay is performed with small amounts of a known ERα agonist (E2) added to each well, and when an antagonistic compound binds to the LBD of the GAL4-ERα fusion protein binding to the UAS is inhibited, interfering with expression of β-lactamase. The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 (Graham et al. 1977). The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid (Bylund et al. 2004). HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
Phenol red-free DMEM	Invitrogen	Invitrogen/21063
DMEM	Invitrogen	Invitrogen/11965
Dialyzed FBS	Invitrogen	Invitrogen/26400
Charcoal stripped FBS	Invitrogen	Invitrogen/12676
NEAA	Invitrogen	Invitrogen/11140
Sodium pyruvate	Invitrogen	Invitrogen/11360
Penicillin and Streptomycin	Invitrogen	Invitrogen/15140
Hygromycin B	Invitrogen	Invitrogen/10687
Zeocin	Invitrogen	Invitrogen/R25001
Recovery Cell Culture Freezing Medium	Invitrogen	Invitrogen/12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen/25300
LiveBLAzer B/G FRET substrate	Invitrogen	Invitrogen/K1028
CellTiter-Glo Assay Custom Solution	Promega	Promega / X2371
Black-clear bottom 1536 well plates	Greiner	Greiner/789092F
BioRAPTR FRD dispenser	Beckman Coulter	Beckman Coulter
Multidrop COMBI	Thermo Electron Corporation	Thermo Electron Corporation
Envision Plate Reader	Perkin Elmer	Perkin Elmer
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
4-Hydroxytamoxifen (Antagonist control compound)	Sigma	Sigma/H7904

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Phenol red-free DMEM	-	98%	-	-
DMEM	90%	-	90%	-
Dialyzed FBS	10%	-	10%	-
Charcoal stripped FBS	-	2%	-	-
NEAA	0.1mM	0.1mM	0.1mM	-
Sodium pyruvate	1mM	1mM	1mM	-
Penn-strep	100U/ml-100µg/ml	100U/ml-100µg/ml	100U/ml-100µg/ml	-

Hygromycin B	80µg/ml	-	-	-
Zeocin	100µg/ml	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 1ml frozen cells of ERalpha-bla were taken in pre-warmed 10ml of thaw medium for centrifuging.

1.2.2 2-3ml of the thaw medium is taken to resuspend the pellet

1.2.3 The cells were seeded in T-75 flask at 2 million cells

1.3. Propagation method

1.3.1 The cells are detached using 0.05% Trypsin

1.3.2 Cells are further passaged at a density of 4-5 million cells per T-225 flask

2. Assay Protocol

2.1 Rinse the cells twice with DPBS and detach them using 0.05% Trypsin and centrifuge

2.2 Resuspend the pellet with assay buffer

2.3 Plate the cells in black-clear bottom 1536 well plate at 5000/well/5µL through 8 tip plate dispenser (Multi drop)

2.4 Incubate at 37°C for 5hrs

2.5 Transfer 23nL of the compounds from the library collection and positive control through Pintool

2.6 Add 1µL of assay buffer with or without 0.5nM (final) Beta-estradiol

2.7 Incubate at 37°C for 18hrs

2.8 Add 1µL of CCF4 dye using a single tip of a plate dispenser (Bioraptr)

2.9 Incubate at room temperature for 2hrs

2.10 Read the fluorescence intensity through Envision plate reader

2.11 Add 4µL of CellTiter-Glo reagent using a single tip of a plate dispenser (Bioraptr)

2.12 Incubate at room temperature for 30min

2.13 Read the luminescence through ViewLux plate reader

The ER-alpha-bla cells were dispensed at 5,000 cells/5 µL of assay medium per well into black wall/clear-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37°C and 5% CO2 for 4 hr, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1 µL of 17β-estradiol in assay medium using a Flying Reagent Dispenser (Aurora Discovery, San Diego, CA). The plates were incubated at 37°C and 5% CO2 for 18 hr. Then 1 µL of LiveBLAzerTM B/G FRET substrate was added using a Flying Reagent Dispenser, the plates were incubated at room temperature for 2 hours, and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 3 µL/well of CellTiter-Glo reagent was added into the assay plates using a FRD. After 30 min incubation at room temperature, the luminescence intensity in the plates was measured using a ViewLux (PerkinElmer) plate reader.

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration,

plate centrifugation and incubation, et cetera) and signal recording (plate readout) (Michael et al. 2008). GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Sakamuru, S., Martin, M. T., Reif, D. M., Judson, R. S., Houck, K. A., Casey, W., Hsieh, J. H., Shockley, K. R., Ceger, P., Fostel, J., Witt, K. L., Tong, W., Rotroff, D. M., Zhao, T., Shinn, P., Simeonov, A., Dix, D. J., Austin, C. P., Kavlock, R. J., Tice, R. R., & Xia, M. (2014). "Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway". Scientific Reports 4, 5664. (PMID: 25012808)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 estrogen receptor alpha beta lactamase antagonist assay screened a library of diverse environmental compounds to probe for xenoestrogenic ligand-binding and potential to suppress estrogen-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. Each well contained 0.5nM β-estradiol as an ER stimulator

and measured the loss-of-signal as compared to positive control of 4-Hydroxytamoxifen (100% inhibition). The assay is run in triplicate on 1536-well microplates. Following 18 hour incubation of cells with test compounds, a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for 2 hours. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (product) to green (substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader and CellTiter-Glo assay reagent (Promega) is also incubated with test system for 30 minutes before readout to detect cell viability.

The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER antagonists, GeneBLAzer® ERα-UAS-bla GripTite™ cell line (Invitrogen) has been used to screen the Tox21 library of diverse environmental compounds. ERα-UAS-bla cell line expresses a partial ERα one-hybrid GAL4 system and is stably transfected with a β-lactamase reporter gene.

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Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that

efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Xenoestrogenic ligand-binding and ERα antagonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (4-hydroxytamoxifen; 100% activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of 4-hydroxytamoxifen activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440

OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.329
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.861
Neutral control median absolute deviation, by plate:	19.331
Positive control well median response value, by plate:	-100.09
Positive control well median absolute deviation, by plate:	2.96
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.31
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-5.13
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	115.14
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-21.07

Reference Chemicals / Predictive Capacity:

3.3. Assay Scope and Limitations

Chemical Library:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate

LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [4].

4. Assay Documentation

4.1. References

[1] Graham, F., et al. (1977). J Gen Virol 36(1): 59-72. (PMID: 886304)

[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)

[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)

[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

E2, Estradiol

EDC, Endocrine Disrupting Compounds

ER, Estrogen Receptor

ERE, Estrogen Response Element

FBS, Fetal Bovine Serum

FRD, Flying Reagent Dispenser

ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

MIE, Molecular Initiating Event

NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods

NR, Nuclear Receptors

qHTS, quantitative high-throughput screening

TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

7 June 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

Tox21_ERa_LUC_BG1_Agonist

Assay Name: Tox21 BG1 Cell-Based qHTS Luciferase Assay to Identify Small Molecule Agonists of the Estrogen Receptor Alpha (ER-alpha) Signaling Pathway

1. Assay Descriptions

1.1. Overview

Assay Summary:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER agonists, BG1-Luc-4E2 cell line has been used to screen the Tox21 library of environmental compounds. The BG1Luc4E2 cell line endogenously expresses full-length ERα and is stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere). This test system was plated into 1536-well microplates and cells were exposed to test chemicals or controls for 24 hours and scanned with a microplate reader to detect bioluminescent signals which result from the enzymatic reaction catalyzed by ONE-Glo™ assay substrate and the induction of an ERα-linked luciferase reporter gene.

1.2. Assay Definition

Assay Throughput:

Stably transfected BG-1 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring luminescence resulting from ER gene expression.

Experimental System:

Bowman Gray-1 (BG-1) cells are an ovarian adenocarcinoma originating from tumor tissue taken in the early 1980's from a patient with poorly differentiated Stage III ovarian cancer. This is an immortalized cell line which endogenously expresses receptors for estrogen (α and β) and progesterone [1] as well as growth factors EGF and IGF-1 [2], and so provides an alternative to breast cell lines for estrogen-sensitive proliferation assays. BG1-Luc-4E2 cells are BG-1 cells which are stably transfected with plasmid containing four estrogen responsive elements upstream of a luciferase reporter gene [3] to measure transactivation activity occurring along estrogen signaling pathways. The BG1luc estrogen receptor transactivation test method for identifying estrogen receptor activation has been validated by NICEATM and ICCVAM as an appropriate assay for detecting ER agonism [4].

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
MEM α medium	Invitrogen	Invitrogen, 12561
10% Premium Fetal Bovine Serum	Atlanta Biologicals	Atlanta Biologicals, S11150
Penicillin/Streptomycin	Invitrogen	Invitrogen, 15140

400mg/l G418 (Geneticin)	Invitrogen	Invitrogen, 10131
DMEM phenol red free - low glucose medium	Invitrogen	Sigma, D5921
10% Charcoal/dextran treated Fetal Bovine Serum	Invitrogen	Invitrogen, 12676
L-Glutamine	Invitrogen	Invitrogen, 25030
0.25% Trypsin-EDTA	Invitrogen	Invitrogen / 25200
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo Luciferase Assay system	Promega	Promega / E6130
Recovery Cell Culture Freezing Medium	Invitrogen	Invitrogen / 12648

Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
MEM α medium	90%	-	90%	-
DMEM phenol red free - low glucose medium	-	90%	-	-
Premium Fetal Bovine Serum	10%	-	10%	-
Charcoal/dextran treated Fetal Bovine Serum	-	10%	-	-
Penicillin/Streptomycin	100U/ml & 100µg/ml	100U/ml & 100µg/ml	100U/ml & 100µg/ml	-
L-Glutamine	-	2mM	-	-
G418 (Geneticin)	400mg/l	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 Thaw a frozen vial of cells in 9ml of pre-warmed medium and seed them in T175 flask at 2 million cells

1.3. Propagation method

1.3.1 Trypsinize cells from the flask and centrifuge

1.3.2 Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2.0 Assay Protocol

2.1 Harvest from the 5-day culture in assay medium and re-suspend cells in assay medium
2.2 Dispense 4000 cells/5µL/well into 1536-well tissue treated white/solid bottom plates
2.3 Incubate the plates for 24hrs (22-24hrs) at 37°C and 5% CO2
2.4 Transfer 23nL of compounds from the library collection and positive control to the assay plates through pintool
2.5 Incubate the plates for 22hrs (22-24hrs) at 37°C and 5% CO2
2.6 Add 5µl of ONE-Glo reagent
2.7 Incubate the plates at room temperature for 30min
2.8 Measure luminescence by ViewLux plate reader

Protocol Summary:

BG1-Luc-4E2 cells were cultured in phenol red-free assay medium containing 10% charcoal stripped FBS for 5 days before the assay was performed. The cells were dispensed at 4,000 cells/5 µL of assay medium per well into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37C and 5% CO2 for 24 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The plates were incubated at 37C and 5% CO2 for 22 h, followed by addition of 5 µL of ONE-Glo reagent (Promega, Madison, WI) to each well using Flying Reagent Dispenser (Aurora Discovery, San Diego, CA). The assay plates were incubated at room temperature and luminescence was measured by a ViewLux plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; BG1-Luc-4E2 cell line was provided by Dr. Michael Denison from University of California. The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [5].

Caveats:

It was recently reported that the BG1-Luc-4E2 cell line was derived from MCF7 human breast cancer cells rather than BG-1 cells. Hence, the cell line has been renamed VM7Luc4E2 (https://ntp.niehs.nih.gov/iccvam/methods/endocrine/bg1luc/bg1luc-vm7luc-june2016-508.pdf). The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]

National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Sakamuru, S., Martin, M. T., Reif, D. M., Judson, R. S., Houck, K. A., Casey, W., Hsieh, J. H., Shockley, K. R., Ceger, P., Fostel, J., Witt, K. L., Tong, W., Rotroff, D. M., Zhao, T., Shinn, P., Simeonov, A., Dix, D. J., Austin, C. P., Kavlock, R. J., Tice, R. R., & Xia, M. (2014). "Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway". Scientific Reports 4, 5664. (PMID: 25012808)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 estrogen receptor alpha BG1 luciferase agonist assay screened a library of diverse environmental compounds to probe for xenoestrogenic ligand-binding and potential to induce estrogen-dependent transcription, monitored through luciferase reporter gene signal activation using an endogenous full-length ERα cell line of human ovary origin (BG1). The assay is run in triplicate on 1536-well microplate and bioluminescence was measured following 24-hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM.

Scientific Principles:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER agonists, BG1-Luc-4E2 cell line (provided by Dr. Michael Denison from University of California) has been used to screen the Tox21 library of diverse environmental compounds. BG1Luc4E2 cell line endogenously expresses full-length ER-alpha and is stably transfected with a plasmid containing four estrogen responsive elements (ERE) upstream of a luciferase reporter gene.

The ERα_LUC_BG1 assays are qHTS format assays which measured the ability of a chemical to interact with estrogen receptor alpha (ERα) by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human ovary (BG1) cell line which expresses endogenous full-length ERα and a luciferase reporter gene (ER-luc) to quantify xenoestrogenic activity**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Rogers, J., & Denison, M. (2000). "Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals". In Vitro Mol Toxicol 13(1), 67-82. (PMID: 10900408)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.18
Neutral control median absolute deviation, by plate:	6.87
Positive control well median response value, by plate:	99.58
Positive control well median absolute deviation, by plate:	8.91
Z' (median across all plates, using positive control wells):	0.53
SSMD (median across all plates, using positive control wells):	9
Signal-to-noise (median across all plates, using positive control wells):	14.55
Signal-to-background (median across all plates, using positive control wells):	-548.47
CV (median across all plates):	-39.74

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Xenoestrogenic ligand-binding and ERα agonism as monitored by measuring changes in luminescence resulting from activation of an estrogen-responsive luciferase reporter gene.

Analytical Elements:

The Tox21 BG1-luciferase ERα agonist assay was monitored for signal increase over the DMSO (negative control) baseline, and activity and was calculated as a percentage of 17β-estradiol (positive control) activity. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized

response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_BG1_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 15
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.001 µM
Standard maximum concentration tested: 90 µM
Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 2.78
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes

58-18-4	17-Methyltestosterone	Very Weak	Active	Yes
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	Yes
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	Yes
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	Yes
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	Yes
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No
84-74-2	Dibutyl phthalate	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	No
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	No
120-47-8	Ethylparaben	Very Weak	NA	No

60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	Yes
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	Yes
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	Yes
57-30-7	Phenobarbital sodium	Inactive	NA	Yes
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	8
Inactive	5	3

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	9
Inactive	7	4

In Vitro Sensitivity = 72.4%
In Vitro Specificity = 37.5%
Balanced Accuracy = 55.0%

In Vivo Sensitivity = 70.0%
In Vivo Specificity = 36.4%
Balanced Accuracy = 53.2%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and

suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [5].

4. Assay Documentation

4.1. References

[1] Geisinger, K. R., et al. (1989). Cancer 63(2): 280-288. (PMID: 2910432)

[2] Baldwin, W. S., et al. (1998). In Vitro Cell Develop Biol -Animal 34(8): 649-654. (PMID: 9769151)

[3] Rogers, J. and M. Denison (2000). In Vitro Mol Toxicol 13(1): 67-82. (PMID: 10900408)

[4] OECD (2012). Test No. 457: Bg1luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists, OECD Publishing.

[5] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

E2, Estradiol

EDC, Endocrine Disrupting Compounds

ER, Estrogen Receptor

ERE, Estrogen Response Element

FBS, Fetal Bovine Serum

FRD, Flying Reagent Dispenser

ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

MIE, Molecular Initiating Event

NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods

NR, Nuclear Receptors

qHTS, quantitative high-throughput screening

TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

3 June 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:
Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation
Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;
Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

Tox21_ERa_LUC_VM7_Agonist_ 10nM_ICI182780

Assay Name: Tox21 VM7 Cell-Based qHTS Luciferase Assay to Identify Small Molecule Agonists of the Estrogen Receptor Alpha (ER-alpha) Signaling Pathway

1. Assay Descriptions

1.1. Overview

Assay Summary:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER agonists, VM7-Luc-4E2 cell line has been used to screen the Tox21 library of environmental compounds. The VM7Luc4E2 cell line endogenously expresses full-length ERα and is stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere). This test system was plated into 1536-well microplates and cells were exposed to test chemicals or controls for 24 hours and scanned with a microplate reader to detect bioluminescent signals which result from the enzymatic reaction catalyzed by ONE-Glo™ assay substrate and the induction of an ERα-linked luciferase reporter gene.

1.2. Assay Definition

Assay Throughput:

Stably transfected VMCF7 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring luminescence resulting from ER gene expression.

Experimental System:

MCF-7 (Michigan Cancer Foundation-7) is a human breast carcinoma cell line originating from tumor tissue taken in 1970 from a 69-year old Caucasian woman. This is an immortalized cell line which endogenously expresses receptors for estrogen (α and β) and progesterone [1] as well as growth factors EGF and IGF-1 [2], and so provides an alternative to breast cell lines for estrogen-sensitive proliferation assays. VM7-Luc-4E2 cells are VMCF7 cells which are stably transfected with plasmids containing four estrogen responsive elements upstream of a luciferase reporter gene [3] to measure transactivation activity occurring along estrogen signaling pathways. The VM7luc estrogen receptor transactivation test method for identifying estrogen receptor activation has been validated by NICEATM and ICCVAM as an appropriate assay for detecting ER antagonism [4].

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
MEM α medium	Invitrogen	Invitrogen, 12561
10% Premium Fetal Bovine Serum	Atlanta Biologicals	Atlanta Biologicals, S11150

Penicillin/Streptomycin	Invitrogen	Invitrogen, 15140
400mg/l G418 (Geneticin)	Invitrogen	Invitrogen, 10131
DMEM phenol red free - low glucose medium	Invitrogen	Sigma, D5921
10% Charcoal/dextran treated Fetal Bovine Serum	Invitrogen	Invitrogen, 12676
L-Glutamine	Invitrogen	Invitrogen, 25030
0.25% Trypsin-EDTA	Invitrogen	Invitrogen / 25200
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo Luciferase Assay system	Promega	Promega / E6130
Recovery Cell Culture Freezing Medium	Invitrogen	Invitrogen / 12648
ICI 182,780	Sigma-Aldrich	Sigma-Aldrich/I4409

Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
MEM α medium	90%	-	90%	-
DMEM phenol red free - low glucose medium	-	90%	-	-
Premium Fetal Bovine Serum	10%	-	10%	-
Charcoal/dextran treated Fetal Bovine Serum	-	10%	-	-
Penicillin/Streptomycin	100U/ml & 100µg/ml	100U/ml & 100µg/ml	100U/ml & 100µg/ml	-
L-Glutamine	-	2mM	-	-
G418 (Geneticin)	400mg/l	-	-	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 Thaw a frozen vial of cells in 9ml of pre-warmed medium and seed them in T175 flask at 2 million cells

1.3. Propagation method

1.3.1 Trypsinize cells from the flask and centrifuge

1.3.2 Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2.0 Assay Protocol

2.1 Harvest from the 5-day culture in assay medium and re-suspend cells in assay medium

2.2 Dispense 4000 cells/4µL/well into 1536-well tissue treated white/solid bottom plates

2.3 Incubate the plates for 24hrs (22-24hrs) at 37°C and 5% CO2

2.4 1 µL of 50.0 nM ICI 182,780/assay medium (10 nM final concentration) was added using a dispenser.

2.5 Transfer 23nL of compounds from the library collection and positive control to the assay plates through pintool

2.6 Incubate the plates for 22hrs (22-24hrs) at 37°C and 5% CO2

2.7 Add 5µl of ONE-Glo reagent

2.8 Incubate the plates at room temperature for 30min

2.9 Measure luminescence by ViewLux plate reader

Protocol Summary:

VM7-Luc-4E2 cells were cultured in phenol red-free assay medium containing 10% charcoal stripped FBS for 5 days before the assay was performed. The cells were dispensed at 4,000 cells/4 µL of assay medium per well into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at a 37C and 5% CO2 for 24 h, 1 µL of 50.0 nM ICI 182,780/assay medium was added using a dispenser. Next, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The plates were incubated at 37C and 5% CO2 for 22 h, followed by addition of 5 µL of ONE-Glo reagent (Promega, Madison, WI) to each well using Flying Reagent Dispenser (Aurora Discovery, San Diego, CA). The assay plates were incubated at room temperature and luminescence was measured by a ViewLux plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; VM7-Luc-4E2 cell line was provided by Dr. Michael Denison from University of California. The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [5].

Caveats:

It was recently reported that the VM7-Luc-4E2 cell line was derived from MCF7 human breast cancer cells rather than VMCF7 cells. Hence, the cell line has been renamed VM7Luc4E2 (https://ntp.niehs.nih.gov/iccvam/methods/endocrine/VM7luc/VM7luc-vm7luc-june2016-508.pdf). The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Sakamuru, S., Martin, M. T., Reif, D. M., Judson, R. S., Houck, K. A., Casey, W., Hsieh, J. H., Shockley, K. R., Ceger, P., Fostel, J., Witt, K. L., Tong, W., Rotroff, D. M., Zhao, T., Shinn, P., Simeonov, A., Dix, D. J., Austin, C. P., Kavlock, R. J., Tice, R. R., & Xia, M. (2014). "Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway". Scientific Reports 4, 5664. (PMID: 25012808)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 estrogen receptor alpha VM7 luciferase agonist assay screened a library of diverse environmental compounds to probe for xenoestrogenic ligand-binding and potential to induce estrogen-dependent transcription, monitored through luciferase reporter gene signal activation using an endogenous full-length ERα cell line of human ovary origin (VM7). The assay is run in triplicate on 1536-well microplate and bioluminescence was measured following 24-hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM.

Scientific Principles:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine

function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER agonists, VM7-Luc-4E2 cell line (provided by Dr. Michael Denison from University of California) has been used to screen the Tox21 library of diverse environmental compounds. VM7Luc4E2 cell line endogenously expresses full-length ER-alpha and is stably transfected with a plasmid containing four estrogen responsive elements (ERE) upstream of a luciferase reporter gene.

The ERα_LUC_VM7 assays are qHTS format assays which measured the ability of a chemical to interact with estrogen receptor alpha (ERα) by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human ovary (VM7) cell line which expresses endogenous full-length ERα and a luciferase reporter gene (ER-luc) to quantify xenoestrogenic activity**.**

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Rogers, J., & Denison, M. (2000). "Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals". In Vitro Mol Toxicol 13(1), 67-82. (PMID: 10900408)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	0.928
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	5.139
Z' (median across all plates, using positive control wells):	0.82
SSMD (median across all plates, using positive control wells):	19
Signal-to-noise (median across all plates, using positive control wells):	107.72
Signal-to-background (median across all plates, using positive control wells):	Inf
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Xenoestrogenic ligand-binding and ERα agonism as monitored by measuring changes in luminescence resulting from activation of an estrogen-responsive luciferase reporter gene.

Analytical Elements:

The Tox21 VM7-luciferase ERα agonist assay was run as counter-screen. This assay is a control that is run in agonist mode but with the inclusion of a potent ER antagonist, ICI 182,780, to block agonist activity. Results should be compared to TOX21_ERa_LUC_VM7_Agonist (AEID 788) which was run in the absence of ICI 182,780. Chemicals active in AEID 788 but not in AEID 2211 may be interpreted as competitive ER agonists. Concentration-response relationships were determined based on a

range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_VM7_Antagonist_0.1nM_E2
Tox21_ERa_LUC_VM7_Antagonist_0.5nM_E2
Tox21_ERa_LUC_VM7_Agonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations: 15
Target (nominal) number of replicates: 3
Standard minimum concentration tested: 0.001 µM
Standard maximum concentration tested: 90 µM

Baseline median absolute deviation for the assay - based on the response values at the 2 lowest tested concentrations (bmad): 0.534
The response cutoff used to derive the hit calls (e.g., 5*bmad, 10*bmad): 20

Reference Chemicals / Predictive Capacity:

CASRN	Chemical Name	*In Vitro* Activity	*In Vivo* Activity	Activity in Assay
57-91-0	17alpha-Estradiol	Moderate	Active	Yes
57-63-6	17alpha-Ethinylestradiol	Strong	Active	Yes
50-28-2	17beta-Estradiol	Strong	Active	Yes
58-18-4	17-Methyltestosterone	Very Weak	Active	Yes
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	NA	Active	Yes
131-56-6	2,4-Dihydroxybenzophenone	NA	Active	Yes
5153-25-3	2-Ethylhexylparaben	NA	Active	Yes
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	Moderate	Active	Yes
80-46-6	4-(2-Methylbutan-2-yl)phenol	NA	Active	Yes
80-09-1	4,4'-Sulfonyldiphenol	NA	Active	Yes
599-64-4	4-Cumylphenol	Weak	Active	Yes
104-43-8	4-Dodecylphenol	NA	Active	Yes
99-96-7	4-Hydroxybenzoic acid	acid	Inactive	Yes
104-40-5	4-Nonylphenol	Very Weak	Active	Yes
98-54-4	4-tert-Butylphenol	NA	Active	Yes
521-18-6	5alpha-Dihydrotestosterone	Weak	Active	Yes
61-82-5	Amitrole	NA	Inactive	Yes
520-36-5	Apigenin	Very Weak	NA	Yes
85-68-7	Benzyl butyl phthalate	Very Weak	NA	Yes
103-23-1	Bis(2-ethylhexyl)hexanedioate	NA	Inactive	Yes
80-05-7	Bisphenol A	Weak	Active	Yes
1478-61-1	Bisphenol AF	NA	Active	Yes
77-40-7	Bisphenol B	Weak	Active	Yes
94-26-8	Butylparaben	NA	Active	Yes
480-40-0	Chrysin	Very Weak	NA	Yes
50-22-6	Corticosterone	Inactive	NA	Yes
486-66-8	Daidzein	Weak	NA	Yes
117-81-7	Di(2-ethylhexyl) phthalate	Very Weak	Inactive	No

84-74-2	Dibutyl phthalate	Very Weak	Inactive	No
115-32-2	Dicofol	Very Weak	NA	Yes
84-61-7	Dicyclohexyl phthalate	NA	Inactive	Yes
84-66-2	Diethyl phthalate	NA	Inactive	No
56-53-1	Diethylstilbestrol	Strong	Active	No
84-75-3	Dihexyl phthalate	NA	Inactive	No
474-86-2	Equilin	NA	Active	Yes
50-27-1	Estriol	NA	Active	Yes
53-16-7	Estrone	Moderate	Active	No
120-47-8	Ethylparaben	Very Weak	NA	No
60168-88-9	Fenarimol	Very Weak	NA	Yes
51630-58-1	Fenvalerate	NA	Inactive	Yes
446-72-0	Genistein	Weak	Active	Yes
52-86-8	Haloperidol	Inactive	NA	Yes
520-18-3	Kaempferol	Very Weak	Inactive	Yes
143-50-0	Kepone	Weak	NA	No
65277-42-1	Ketoconazole	Inactive	NA	No
330-55-2	Linuron	Inactive	NA	Yes
84-16-2	meso-Hexestrol	Strong	NA	Yes
72-33-3	Mestranol	NA	Active	Yes
72-43-5	Methoxychlor	Very Weak	Active	Yes
68-22-4	Norethindrone	NA	Active	Yes
789-02-6	o,p'-DDT	Weak	Active	Yes
556-67-2	Octamethylcyclotetrasiloxane	NA	Active	Yes
72-55-9	p,p'-DDE	Very Weak	Weak	Yes
87-86-5	Pentachlorophenol	NA	Inactive	Yes
57-30-7	Phenobarbital sodium	Inactive	NA	Yes
32809-16-8	Procymidone	Inactive	NA	No
50-55-5	Reserpine	Inactive	NA	No
52-01-7	Spironolactone	Inactive	NA	No
17924-92-4	Zearalenone	NA	Active	Yes

In Vitro Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	8
Inactive	5	3

In Vivo Activity	**ToxCast Active**	**ToxCast Inactive**
Active	21	9
Inactive	7	4

In Vitro Sensitivity = 72.4%
In Vitro Specificity = 37.5%
Balanced Accuracy = 55.0%

In Vivo Sensitivity = 70.0%

In Vivo Specificity = 36.4%
Balanced Accuracy = 53.2%

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [5].

4. Assay Documentation

4.1. References

[1] Geisinger, K. R., et al. (1989). Cancer 63(2): 280-288. (PMID: 2910432)
[2] Baldwin, W. S., et al. (1998). In Vitro Cell Develop Biol -Animal 34(8): 649-654. (PMID: 9769151)
[3] Rogers, J. and M. Denison (2000). In Vitro Mol Toxicol 13(1): 67-82. (PMID: 10900408)
[4] OECD (2012). Test No. 457: VM7luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists, OECD Publishing.
[5] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
E2, Estradiol
EDC, Endocrine Disrupting Compounds
ER, Estrogen Receptor
ERE, Estrogen Response Element
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, quantitative high-throughput screening
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

3 June 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Tox21_ERa_LUC_VM7_Antagonist_0.1nM_E2

Assay Name: Tox21 VM7 Cell-Based qHTS Luciferase Assay to Identify Small Molecule Agonists of the Estrogen Receptor Alpha (ER-alpha) Signaling Pathway

1. Assay Descriptions

1.1. Overview

Assay Summary:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER antagonists, VM7-Luc-4E2 cell line has been used to screen the Tox21 library of environmental compounds. The VM7Luc4E2 cell line endogenously expresses full-length ERα and is stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere). To measure antagonistic activity, this assay is performed with small amounts of an ERα stimulator (β-estradiol) added to each well and each compound is evaluated against a known ERα antagonist (4-Hydroxytamoxifen) as a positive control (100% inhibition). This test system was plated into 1536-well microplates and cells were exposed to test chemicals or controls for 24 hours and scanned with a microplate reader to detect bioluminescent signals which result from the enzymatic reaction catalyzed by ONE-Glo™ assay substrate and the induction of an ERα-linked luciferase reporter gene. To detect loss of signal due to compound cytotoxicity, a CellTiter-Glo fluorescence assay to measure ATP production was run concurrently in all wells using tetraoctylammonium bromide as a positive control for cell death. The antagonist assay was also run with a higher concentration of agonist (AEID789) as a specificity control in which competitive antagonists should show a right-shift in potency relative to the assay with lower agonist concentration.

1.2. Assay Definition

Assay Throughput:

Stably transfected VMCF7 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring luminescence resulting from ER gene expression.

Experimental System:

MCF-7 (Michigan Cancer Foundation-7) is a human breast carcinoma cell line originating from tumor tissue taken in 1970 from a 69-year old Caucasian woman. This is an immortalized cell line which endogenously expresses receptors for estrogen (α and β) and progesterone [1] as well as growth factors EGF and IGF-1 [2], and so provides an alternative to breast cell lines for estrogen-sensitive proliferation assays. VM7-Luc-4E2 cells are VMCF7 cells which are stably transfected with plasmids containing four estrogen responsive elements upstream of a luciferase reporter gene [3] to measure transactivation activity occurring along estrogen signaling pathways. The VM7luc estrogen receptor transactivation test method for identifying estrogen receptor activation has been validated by NICEATM and ICCVAM as an appropriate assay for detecting ER antagonism [4].

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

QUALITY CONTROL PRECAUTIONS:

1. -Maintain cells below 85-90% confluence in culture medium
2. -For assay purpose, the cells should be cultured in assay medium with 10% charcoal treated FBS for 5 days with alternate day medium changed to fresh medium
3. -Especially while in assay culture, the cells should not reach more than 85% confluence as they would become harder to detach if they reach over confluence

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
MEM α medium	Invitrogen	Invitrogen, 12561
10% Premium Fetal Bovine Serum	Atlanta Biologicals	Atlanta Biologicals, S11150
Penicillin/Streptomycin	Invitrogen	Invitrogen, 15140
400mg/l G418 (Geneticin)	Invitrogen	Invitrogen, 10131
DMEM phenol red free - low glucose medium	Invitrogen	Sigma, D5921
10% Charcoal/dextran treated Fetal Bovine Serum	Invitrogen	Invitrogen, 12676
L-Glutamine	Invitrogen	Invitrogen, 25030
0.25% Trypsin-EDTA	Invitrogen	Invitrogen / 25200
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo Luciferase Assay system	Promega	Promega / E6130
Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
CellTiter-Fluor Cell Viability Assay	Promega	Promega / G6082

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
MEM α medium	90%	-	90%	-
DMEM phenol red free - low glucose medium	-	90%	-	-
Premium Fetal Bovine Serum	10%	-	10%	-
Charcoal/dextran treated Fetal Bovine Serum	-	10%	-	-

Penicillin/Streptomycin	100U/ml & 100µg/ml	100U/ml & 100µg/ml	100U/ml & 100µg/ml	-
L-Glutamine	-	2mM	-	-
G418 (Geneticin)	400mg/l	-	-	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 Thaw a frozen vial of cells in 9ml of pre-warmed medium and seed them in T175 flask at 2 million cells

1.3. Propagation method

1.3.1 Trypsinize cells from the flask and centrifuge

1.3.2 Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2.0 Assay Protocol

2.1 Harvest cells from the 5-day culture in assay medium and resuspend cells in assay medium

2.2 Dispense 4000 cells/5µL/well into 1536-well tissue treated white/solid bottom plates

2.3 Incubate the plates for 24hrs (22-24hrs) at 37°C and 5% CO2

2.4 Transfer 23nL of compounds from the library collection and positive control to the assay plates through pintool

2.5 Add 1µL of 0.5 nM (0.1 nM final) beta-Estradiol (E2, agonist) or assay buffer

2.6 Incubate the plates for 22-24hrs at 37°C and 5% CO2

2.7 Add 1ul of CellTiter-Fluor reagent

2.8 Incubate the plates at 37°C for 30min

2.9 Measure fluorescence by ViewLux plate reader

2.10 Add 4ul of ONE-Glo reagent

2.11 Incubate the plates at room temperature for 30min

2.12 Measure luminescence by ViewLux plate reader

Protocol Summary:

VM7-Luc-4E2 cells were cultured in phenol red free assay medium containing 10% charcoal stripped FBS for 5 days before the assay was performed. The cells were dispensed at 4,000 cells/4 µL of assay medium per well into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. The assay plates were incubated at a 37°C and 5% CO2 for 24 h, and then 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate using a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1 µL of 17β-estradiol in assay medium using a Flying Reagent Dispenser (Aurora Discovery, San Diego, CA). After the plates were incubated at 37°C and 5% CO2 for 21.5 h, 1 µL of CellTiter-Fluor reagent (Promega, Madison, WI) of measuring cytotoxicity was added using Flying Reagent Dispenser (FRD) to each well. The assay plates were incubated at 37°C and 5% CO2 for additional 30 min and the fluorescence intensity was quantified by a ViewLux plate reader (PerkinElmer, Shelton, CT). For measuring luciferase reporter gene activity, 4 µL of ONE-Glo reagent (Promega) was added to each plate using an FRD and luminescence was quantified by a ViewLux plate reader after 30 min incubation at room temperature.

Proprietary Elements:

This assay is not proprietary; VM7-Luc-4E2 cell line was provided by Dr. Michael Denison from University of California. The Tox21 qHTS robotic platform has a 1536-well per run capacity and is

capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [5].

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Sakamuru, S., Martin, M. T., Reif, D. M., Judson, R. S., Houck, K. A., Casey, W., Hsieh, J. H., Shockley, K. R., Ceger, P., Fostel, J., Witt, K. L., Tong, W., Rotroff, D. M., Zhao, T., Shinn, P., Simeonov, A., Dix, D. J., Austin, C. P., Kavlock, R. J., Tice, R. R., & Xia, M. (2014). "Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway". Scientific Reports 4, 5664. (PMID: 25012808)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 estrogen receptor alpha VM7 luciferase antagonist assay screened a library of diverse environmental compounds to probe for xenoestrogenic ligand-binding and potential to inhibit estrogen-dependent transcription, monitored through luciferase reporter gene signal activity using an endogenous full-length ERα cell line of human ovary origin (VM7). The assay is run in triplicate on 1536-well microplate and bioluminescence was measured following 24 hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were assayed for cytotoxicity by measuring protease activity with Promega CellTiter-

Fluor with tetraoctylammonium bromide as a positive control for cell death. The compound library was selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. To help distinguish true antagonistic activity from cytotoxic effects, this assay was multiplexed with a fluorescence-based cell viability assay which measured conserved and constitutive protease activity within live cells (Promega). Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER antagonists, VM7-Luc-4E2 cell line (provided by Dr. Michael Denison from University of California) has been used to screen the Tox21 library of diverse environmental compounds. VM7Luc4E2 cell line endogenously expresses full-length ER-alpha and is stably transfected with a plasmid containing four estrogen responsive elements (ERE) upstream of a luciferase reporter gene.

The ERα_LUC_VM7 assays are qHTS format assays which measured the ability of a chemical to interfere with estrogen receptor alpha (ERα) by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human breast carcinoma (vMCF7) cell line which expresses endogenous full-length ERα and a luciferase reporter gene (ER-luc) to quantify xenoestrogenic activity**.** The cell line was previously thought to be an ovarian cancer line (BG-1). This change does not alter published validation studies. Additional documentation on this change can be found here: https://ntp.niehs.nih.gov/iccvam/methods/endocrine/VM7luc/VM7luc-vm7luc-june2016-508.pdf.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Rogers, J., & Denison, M. (2000). "Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals". In Vitro Mol Toxicol 13(1), 67-82. (PMID: 10900408)

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.815
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.344
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.75
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-14.67
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Xenoestrogenic ligand-binding and ERα antagonism as monitored by measuring changes in luminescence resulting from activation of an estrogen-responsive luciferase reporter gene.

Analytical Elements:

The Tox21 VM7-luciferase ERα antagonist assay was monitored for decreased activity (loss-of-signal) relative to DMSO (negative control) and 4-hydroxytamoxifen (positive control, 100% antagonist activity) measured in the presence of 0.1 nM estradiol agonist stimulation. This assay was also run with 0.5 nM E2 (AEID 789) which can be used to distinguish competitive antagonists from non-competitive or cytotoxic chemicals. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
Tox21_ERa_BLA_Agonist_ratio
Tox21_ERa_BLA_Antagonist_ratio
Tox21_ERa_LUC_VM7_Agonist
Tox21_ERa_LUC_VM7_Agonist_10nM_ICI182780
Tox21_ERa_LUC_VM7_Antagonist_0.5nM_E2

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.927
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.56

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure

potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [5].

4. Assay Documentation

4.1. References

[1] Geisinger, K. R., et al. (1989). Cancer 63(2): 280-288. (PMID: 2910432)

[2] Baldwin, W. S., et al. (1998). In Vitro Cell Develop Biol -Animal 34(8): 649-654. (PMID: 9769151)

[3] Rogers, J. and M. Denison (2000). In Vitro Mol Toxicol 13(1): 67-82. (PMID: 10900408)

[4] OECD (2012). Test No. 457: VM7luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists, OECD Publishing.

[5] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

E2, Estradiol

EDC, Endocrine Disrupting Compounds

ER, Estrogen Receptor

ERE, Estrogen Response Element

FBS, Fetal Bovine Serum

FRD, Flying Reagent Dispenser

ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

MIE, Molecular Initiating Event

NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods

NR, Nuclear Receptors

qHTS, quantitative high-throughput screening

TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

3 June 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (see existing annotations)

Tox21_ERa_LUC_VM7_Antagonist_0.5nM_E2

Assay Name: Tox21 VM7 Cell-Based qHTS Luciferase Assay to Identify Small Molecule Agonists of the Estrogen Receptor Alpha (ER-alpha) Signaling Pathway

1. Assay Descriptions

1.1. Overview

Assay Summary:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER antagonists, VM7-Luc-4E2 cell line has been used to screen the Tox21 library of environmental compounds. The VM7Luc4E2 cell line endogenously expresses full-length ERα and is stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere). To measure antagonistic activity, this assay is performed with small amounts of an ERα stimulator (β-estradiol) added to each well and each compound is evaluated against a known ERα antagonist (4-Hydroxytamoxifen) as a positive control (100% inhibition). This test system was plated into 1536-well microplates and cells were exposed to test chemicals or controls for 24 hours and scanned with a microplate reader to detect bioluminescent signals which result from the enzymatic reaction catalyzed by ONE-Glo™ assay substrate and the induction of an ERα-linked luciferase reporter gene. To detect loss of signal due to compound cytotoxicity, a CellTiter-Glo fluorescence assay to measure ATP production was run concurrently in all wells using tetraoctylammonium bromide as a positive control for cell death. This assay serves as a specificity control for AEID 2053 through use of a higher agonist concentration which should result in a right shift in potency for competitive AR antagonists relative to the lower agonist concentration used in AEID 2053.

1.2. Assay Definition

Assay Throughput:

Stably transfected VMCF7 cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring luminescence resulting from ER gene expression.

Experimental System:

MCF-7 (Michigan Cancer Foundation-7) is a human breast carcinoma cell line originating from tumor tissue taken in 1970 from a 69-year old Caucasian woman. This is an immortalized cell line which endogenously expresses receptors for estrogen (α and β) and progesterone [1] as well as growth factors EGF and IGF-1 [2], and so provides an alternative to breast cell lines for estrogen-sensitive proliferation assays. VM7-Luc-4E2 cells are vMCF7 cells which are stably transfected with plasmids containing four estrogen responsive elements upstream of a luciferase reporter gene [3] to measure transactivation activity occurring along estrogen signaling pathways. The VM7luc estrogen receptor transactivation test method for identifying estrogen receptor activation has been validated by NICEATM and ICCVAM as an appropriate assay for detecting ER antagonism [4].

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

QUALITY CONTROL PRECAUTIONS:

1. -Maintain cells below 85-90% confluence in culture medium
2. -For assay purpose, the cells should be cultured in assay medium with 10% charcoal treated FBS for 5 days with alternate day medium changed to fresh medium
3. -Especially while in assay culture, the cells should not reach more than 85% confluence as they would become harder to detach if they reach over confluence

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
MEM α medium	Invitrogen	Invitrogen, 12561
10% Premium Fetal Bovine Serum	Atlanta Biologicals	Atlanta Biologicals, S11150
Penicillin/Streptomycin	Invitrogen	Invitrogen, 15140
400mg/l G418 (Geneticin)	Invitrogen	Invitrogen, 10131
DMEM phenol red free - low glucose medium	Invitrogen	Sigma, D5921
10% Charcoal/dextran treated Fetal Bovine Serum	Invitrogen	Invitrogen, 12676
L-Glutamine	Invitrogen	Invitrogen, 25030
0.25% Trypsin-EDTA	Invitrogen	Invitrogen / 25200
1536-well white solid plates	Greiner Bio-One	Greiner Bio-One / 789173-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
ViewLux Plate Reader	Perkin Elmer	Perkin Elmer
ONE-Glo Luciferase Assay system	Promega	Promega / E6130
Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
CellTiter-Fluor Cell Viability Assay	Promega	Promega / G6082

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
MEM α medium	90%	-	90%	-
DMEM phenol red free - low glucose medium	-	90%	-	-
Premium Fetal Bovine Serum	10%	-	10%	-
Charcoal/dextran treated Fetal Bovine Serum	-	10%	-	-

Penicillin/Streptomycin	100U/ml & 100µg/ml	100U/ml & 100µg/ml	100U/ml & 100µg/ml	-
L-Glutamine	-	2mM	-	-
G418 (Geneticin)	400mg/l	-	-	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 Thaw a frozen vial of cells in 9ml of pre-warmed medium and seed them in T175 flask at 2 million cells

1.3. Propagation method

1.3.1 Trypsinize cells from the flask and centrifuge

1.3.2 Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2.0 Assay Protocol

2.1 Harvest cells from the 5-day culture in assay medium and resuspend cells in assay medium

2.2 Dispense 4000 cells/5µL/well into 1536-well tissue treated white/solid bottom plates

2.3 Incubate the plates for 24hrs (22-24hrs) at 37°C and 5% CO2

2.4 Transfer 23nL of compounds from the library collection and positive control to the assay plates through pintool

2.5 Add 1µL of 2.5nM (0.5 nM final) Beta-Estradiol (E2, agonist) or assay buffer

2.6 Incubate the plates for 21.1/2hrs (22-24hrs) at 37°C and 5% CO2

2.7 Add 1ul of CellTiter-Fluor reagent

2.8 Incubate the plates at 37°C for 30min

2.9 Measure fluorescence by ViewLux plate reader

2.10 Add 4ul of ONE-Glo reagent

2.11 Incubate the plates at room temperature for 30min

2.12 Measure luminescence by ViewLux plate reader

Protocol Summary:

VM7-Luc-4E2 cells were cultured in phenol red free assay medium containing 10% charcoal stripped FBS for 5 days before the assay was performed. The cells were dispensed at 4,000 cells/4 µL of assay medium per well into white wall/solid-bottom 1536-well plates using a Multidrop Combi (ThermoFisher Scientific, Waltham, MA) dispenser. The assay plates were incubated at a 37°C and 5% CO2 for 24 h, and then 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate using a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1 µL of 17β-estradiol in assay medium using a Flying Reagent Dispenser (Aurora Discovery, San Diego, CA). After the plates were incubated at 37°C and 5% CO2 for 21.5 h, 1 µL of CellTiter-Fluor reagent (Promega, Madison, WI) of measuring cytotoxicity was added using Flying Reagent Dispenser (FRD) to each well. The assay plates were incubated at 37°C and 5% CO2 for additional 30 min and the fluorescence intensity was quantified by a ViewLux plate reader (PerkinElmer, Shelton, CT). For measuring luciferase reporter gene activity, 4 µL of ONE-Glo reagent (Promega) was added to each plate using an FRD and luminescence was quantified by a ViewLux plate reader after 30 min incubation at room temperature.

Proprietary Elements:

This assay is not proprietary; VM7-Luc-4E2 cell line was provided by Dr. Michael Denison from University of California. The Tox21 qHTS robotic platform has a 1536-well per run capacity and is

capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [5].

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote estrogen receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Huang, R., Sakamuru, S., Martin, M. T., Reif, D. M., Judson, R. S., Houck, K. A., Casey, W., Hsieh, J. H., Shockley, K. R., Ceger, P., Fostel, J., Witt, K. L., Tong, W., Rotroff, D. M., Zhao, T., Shinn, P., Simeonov, A., Dix, D. J., Austin, C. P., Kavlock, R. J., Tice, R. R., & Xia, M. (2014). "Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway". Scientific Reports 4, 5664. (PMID: 25012808)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 estrogen receptor alpha VM7 luciferase antagonist assay screened a library of diverse environmental compounds to probe for xenoestrogenic ligand-binding and potential to inhibit estrogen-dependent transcription, monitored through luciferase reporter gene signal activity using an endogenous full-length ERα cell line of human ovary origin (VM7). This assay was run as a counter-screen to TOX21_ERa_LUC_VM7_Antagonist_0.1nM_E2 (AEID 2053). Results should be compared and competitive antagonists should show a right-shift in potency in AEID 789 relative to AEID 2053. The assay is run in triplicate on 1536-well microplate and bioluminescence was measured following 24 hour incubation of cells with test compounds and 30 min incubation of test

system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were assayed for cytotoxicity by measuring protease activity with Promega CellTiter-Fluor with tetraoctylammonium bromide as a positive control for cell death. The compound library was selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. To help distinguish true antagonistic activity from cytotoxic effects, this assay was multiplexed with a fluorescence-based cell viability assay which measured conserved and constitutive protease activity within live cells (Promega). The assay serves as a specificity control for AEID 2053 as competitive antagonists for the androgen receptor should show a right shift in potency in this assay compared to AEID 2053 due to the use of higher concentration of agonist stimulation.

Scientific Principles:

Estrogen receptor (ER), a nuclear hormone receptor, plays an important role in development, metabolic homeostasis and reproduction. Endocrine disrupting chemicals (EDCs) and their interactions with steroid hormone receptors like ER causes disruption of normal endocrine function. Therefore, it is important to understand the effect of environmental chemicals on the ER signaling pathway. To identify ER antagonists, VM7-Luc-4E2 cell line (provided by Dr. Michael Denison from University of California) has been used to screen the Tox21 library of diverse environmental compounds. VM7Luc4E2 cell line endogenously expresses full-length ER-alpha and is stably transfected with a plasmid containing four estrogen responsive elements (ERE) upstream of a luciferase reporter gene.

The ERα_LUC_VM7 assays are qHTS format assays which measured the ability of a chemical to interfere with estrogen receptor alpha (ERα) by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human breast carcinoma (vMCF7) cell line which expresses endogenous full-length ERα and a luciferase reporter gene (ER-luc) to quantify xenoestrogenic activity**.** The cell line was previously thought to be an ovarian cancer line (BG-1). This change does not alter published validation studies. Additional documentation on this change can be found here: https://ntp.niehs.nih.gov/iccvam/methods/endocrine/VM7luc/VM7luc-vm7luc-june2016-508.pdf.

This assay is intended for use as a part of an integrated testing strategy, to screen a large structurally diverse chemical library for compounds with the potential to interact with estrogen receptor alpha mediated pathways and potentially affect endocrine systems in exposed populations. There is strong evidence that estrogen receptor agonism is the Molecular Initiating Event (MIE) in an Adverse Outcome Pathway (AOP) leading to reproductive dysfunction in oviparous vertebrates, and there is some evidence that estrogen receptor activation is the MIE for putative AOPs leading to reduced survival due to renal failure and leading to skewed sex ratios due to altered sexual differentiation in males (all AOPs currently under development). Chemical-activity profiles derived from this assay can inform prioritization decisions for compound selection in more resource intensive in vivo studies to further investigate the involvement of ER agonism in pathways leading to hazardous outcomes in biological systems.

Method Development Reference:

Rogers, J., & Denison, M. (2000). "Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals". In Vitro Mol Toxicol 13(1), 67-82. (PMID: 10900408)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.216
Neutral control median absolute deviation, by plate:	7.325
Positive control well median response value, by plate:	-100.054
Positive control well median absolute deviation, by plate:	0.902
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.75
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-13.64
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	462.2
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-35.2

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Xenoestrogenic ligand-binding and ERα antagonism as monitored by measuring changes in luminescence resulting from activation of an estrogen-responsive luciferase reporter gene.

Analytical Elements:

The Tox21 VM7-luciferase ERα antagonist specificity assay was monitored for decreased activity (loss-of-signal) relative to DMSO (negative control) and 4-hydroxytamoxifen (positive control, 100% antagonist activity) measured in the presence of 0.5 nM estradiol agonist stimulation. Use of the 0.5 nM estradiol should result in a right-shifting of AC50s for competitive ER antagonists relative to the AEID 2053 assay results run with 0.1 nM estradiol. This is useful for distinguishing competitive antagonists from non-competitive or cytotoxic compounds. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Estrogen receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also

generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ACEA_T47D_80hr_Positive
ATG_ERE_CIS_up
ATG_ERa_TRANS_up
ATG_ERb_TRANS2_up
NVS_NR_bER
NVS_NR_hER
NVS_NR_mERa
OT_ER_ERaERa_0480
OT_ER_ERaERa_1440
OT_ER_ERaERb_0480
OT_ER_ERaERb_1440
OT_ER_ERbERb_0480
OT_ER_ERbERb_1440
OT_ERa_ERELUC_AG_1440
OT_ERa_ERELUC_ANT_1440
OT_ERa_EREGFP_0120
OT_ERa_EREGFP_0480
OT_ERb_ERELUC_ANT_1440
TOX21_ERa_BLA_Agonist_ratio
TOX21_ERa_BLA_Antagonist_ratio
TOX21_ERa_LUC_VM7_Agonist
TOX21_ERa_LUC_VM7_Agonist_10nM_ICI182780

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.083
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate

LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [5].

4. Assay Documentation

4.1. References

[1] Geisinger, K. R., et al. (1989). Cancer 63(2): 280-288. (PMID: 2910432)

[2] Baldwin, W. S., et al. (1998). In Vitro Cell Develop Biol -Animal 34(8): 649-654. (PMID: 9769151)

[3] Rogers, J. and M. Denison (2000). In Vitro Mol Toxicol 13(1): 67-82. (PMID: 10900408)

[4] OECD (2012). Test No. 457: VM7luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists, OECD Publishing.

[5] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

E2, Estradiol

EDC, Endocrine Disrupting Compounds

ER, Estrogen Receptor

ERE, Estrogen Response Element

FBS, Fetal Bovine Serum

FRD, Flying Reagent Dispenser

ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

MIE, Molecular Initiating Event

NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods

NR, Nuclear Receptors

qHTS, quantitative high-throughput screening

TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

3 June 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation
Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;
Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (see existing annotations)

Tox21_FXR_BLA_agonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Peroxisome Farnesoid X (FXR) Agonist qHTS Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The farnesoid-X-receptor (FXR), a ligand-activated nuclear hormone receptor, is highly expressed in liver, intestine, kidney and adrenal cortex. Natural ligands of FXR are the bile acids (e.g., cholic acid, chenodeoxy cholic acid). FXR is an important regulator of diverse metabolic pathways, including bile acid homeostasis, lipid and glucose metabolism. To identify compounds that activate FXR signaling, GeneBLAzer FXR-UAS-bla HEK 293T cell line (Invitrogen, Carlsbad, CA) containing a beta-lactamase reporter gene under the control of a UAS response element was used to screen the Tox21 compound library. This experimental system expresses a fusion protein of a human farnesoid X receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds stimulates β-lactamase reporter gene expression. FXR activation by test compounds was assessed following 24-hour incubation in 1536-well microtiter plates. The cytotoxicity of the Tox21 compound library against the FXR-bla cell line was tested in parallel by measuring the cell viability using CellTiter-Glo assay in the same wells.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from induced FXR gene expression.

Experimental System:

GeneBLAzer® FXR-UAS-bla HEK293T cells contain the ligand-binding domain (LBD) of the human farnesoid X receptor fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-bla HEK293T cell line. GeneBLAzer® UAS-bla HEK 293T cells (Invitrogen catalog #K1104) stably express a beta-lactamase reporter gene under the transcriptional control of an Upstream Activator Sequence (UAS). When an agonist binds to the LBD of the GAL4(DBD)-FXR(LBD) fusion protein, it translocates to the nucleus where it binds to the UAS inducing transcription of beta-lactamase. When an agonist binds to the LBD of the GAL4 (DBD)-FXR (LBD) fusion protein, the protein binds to the UAS, resulting in increased expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM, with Glutamax	Invitrogen	10569
Phenol Red free DMEM	Invitrogen	21063
Dialyzed FBS	Invitrogen	26400
Nonessential amino acids (NEAA)	Invitrogen	11140
DPBS	Invitrogen	14190
Sodium Pyruvate	Invitrogen	11360
Penicillin/Streptomycin	Invitrogen	15140
HEPES (1M pH 7.3)	Invitrogen	15630
0.05% Trypsin/EDTA	Invitrogen	25300
Hygromycin	Invitrogen	10687
Zeocin	Invitrogen	R25001
LiveBLAzer FRET B/G Loading Kit: Solution A, B, and C	Invitrogen	K1030
Recovery Cell Culture Freezing Medium	Invitrogen	12648
Fetal bovine serum (FBS), charcoal stripped	Invitrogen	12676
Chenodeoxycholic acid (CDCA)	Sigma	C9377
Black, clear-bottom, 1536-well assay plates	Greiner BioOne	789092-F
PinTool	Kalypsys	
BioRAPTR, Microfluidic Workstation	Beckmen	
EnVision plate reader	Perkin Elmer	

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Freezing Medium	-	-	-	100%
DMEM+Glutamax	90%	-	90%	-
Phenol Red free DMEM	-	98%	-	-
Dialyzed FBS	10%	-	10%	-
Charcoal-Stripped FBS	-	2%	-	-
Penicillin/Streptomycin	100U/mL;100 µg/mL	100U/mL;100 µg/mL	100U/mL;100 µg/mL	-
Sodium Pyruvate	-	1 mM	-	-
NEAA	0.1 mM	0.1 mM	0.1 mM	-
HEPES (pH 7.3)	25 mM	-	25 mM	-

Hygromycin	100 µg/ml	-	-	-
Zeocin	100 µg/ml	-	-	-

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube

1.2.2 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.

1.2.4 Transfer the vial contents drop-wise into 14 mL of thaw medium in a sterile 15-mL conical tub.

1.2.5 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.

1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37 °C/5% CO2 incubator.

1.2.7 Switch to growth medium at first passage.

1.3. Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add 0.05% Trypsin/EDTA and swirl to coat the cell evenly.

1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.

1.3.3 Centrifuge cells at 900 rpm for 4 minutes and resuspend in growth medium.

1.3.4 Cell should be passage at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture and resuspend in assay medium.

2.2 Dispense 5000 cells/5µL/well into 1536-well tissue treated black, clear-bottom plates using a Multi-drop dispenser.

2.3 After the cells were incubated at 37°C for 5 hours, 23 nL of control or compounds dissolved in DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution.

2.4 Incubate the plates for 16 hours at 37°C.

2.5 Add 1 µL of 6X LiveBLAzer-FRET B/G (CCF4-AM) Substrate Mixture to each well using a BioRAPTR dispenser.

2.6 After two hours incubation at room temperature, measure fluorescence intensity at 460 and 530 nm emission and 405 nm excitation by an Envision detector. Data is expressed as the ratio of 460nm/530nm emissions.

Protocol Summary

FXR-bla cells were dispensed at 5000 cells/5uL/well in 1536-well black wall/clear bottom plates using a Multidrop Combi (Thermo Fisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 C and 5% CO2 for 5 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The assay plates were incubated at 37C for 16 h. After 1 uL of LiveBLAzer B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN), the plates were incubated at room temperature for 2 h, and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 4uL/well of CellTiter-Glo reagent was added into the assay

plates using a Bioraptr FRD. After 30 min incubation at room temperature, the luminescence intensity in the plates was measured using a ViewLux plate reader (PerkinElmer)

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [3]. GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote FXR mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 farnesoid-X-receptor β-lactamase agonist assay screened a library of diverse environmental compounds to probe for xenobiotic ligand-binding and potential to induce FXR-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 16-hour incubation of cells with test compounds a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for an additional hour, in the dark. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Farnesoid-X-receptor (FXR) is a ligand-activated nuclear receptor which regulates the expression of genes involved in bile acid homeostasis and has a role in the regulation of glucose and lipid metabolic pathways. FXR is primarily expressed in the liver, kidney, intestine and adrenal cortex, and regulates the expression of target genes by binding either as a monomer or as a heterodimer with the retinoid X receptor (RXR). Numerous studies have reported that FXR exerts protective function during cholestasis, diabetes, liver regeneration, and cancer. The FXR-RXR heterodimer, when bound to DNA, can act as transcriptional activators or inhibitors. FXR is activated by bile acids and the main endogenous ligand for FXR is chenodeoxycholic acid (CDCA). FXR reduces bile acid concentration in the liver by repressing genes involved in bile acid synthesis and regulates lipid metabolism.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0890
Neutral control median absolute deviation, by plate:	2.638
Positive control well median response value, by plate:	99.490
Positive control well median absolute deviation, by plate:	16.56
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.43
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	6

SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	37.49
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-1026.38
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-27.69

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

FXR ligand-binding and agonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the FXR_BLA_Agonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (Chenodeoxycholic acid, CDCA; 100% agonist activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of CDCA activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up
ATG_FXR_TRANS_up
NVS_NR_hFXR_Agonist
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_0480
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3

Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.720
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [4].

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

ATCC, American Tissue Culture Collection
AIC, Akaike Information Criterion
CDCA, Chenodeoxycholic acid
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
FXR, Farnesoid X receptor
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 October 2016

Date of Revisions:

2016

Author of Revisions:

EPA NCCT

5. Supporting Information (existing annotations):

Tox21_FXR_BLA_antagonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Farnesoid X (FXR) Antagonist qHTS Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The farnesoid-X-receptor (FXR), a ligand-activated nuclear hormone receptor, is highly expressed in liver, intestine, kidney and adrenal cortex. Natural ligands of FXR are the bile acids (e.g., cholic acid, chenodeoxy cholic acid). FXR is an important regulator of diverse metabolic pathways, including bile acid homeostasis, lipid and glucose metabolism. To identify compounds that inhibit FXR signaling, GeneBLAzer FXR-UAS-bla HEK 293T cell line (Invitrogen, Carlsbad, CA) containing a beta-lactamase reporter gene under the control of a UAS response element was used to screen the Tox21 compound library. This experimental system expresses a fusion protein of a human farnesoid X receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds stimulates β-lactamase reporter gene expression. FXR interference by test compounds was assessed following 24-hour incubation in 1536-well microtiter plates. The cytotoxicity of the Tox21 compound library against the FXR-bla cell line was tested in parallel by measuring the cell viability using CellTiter-Glo assay in the same wells.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from induced FXR gene expression.

Experimental System:

GeneBLAzer® FXR-UAS-bla HEK293T cells contain the ligand-binding domain (LBD) of the human farnesoid X receptor fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-bla HEK293T cell line. GeneBLAzer® UAS-bla HEK 293T cells (Invitrogen catalog #K1104) stably express a beta-lactamase reporter gene under the transcriptional control of an Upstream Activator Sequence (UAS). When an agonist binds to the LBD of the GAL4(DBD)-FXR(LBD) fusion protein, it translocates to the nucleus where it binds to the UAS inducing transcription of beta-lactamase. When an agonist binds to the LBD of the GAL4 (DBD)-FXR (LBD) fusion protein, the protein binds to the UAS, resulting in increased expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number

DMEM, with Glutamax	Invitrogen	10569
Phenol Red free DMEM	Invitrogen	21063
Dialyzed FBS	Invitrogen	26400
Nonessential amino acids (NEAA)	Invitrogen	11140
DPBS	Invitrogen	14190
Sodium Pyruvate	Invitrogen	11360
Penicillin/Streptomycin	Invitrogen	15140
HEPES (1M pH 7.3)	Invitrogen	15630
0.05% Trypsin/EDTA	Invitrogen	25300
Hygromycin	Invitrogen	10687
Zeocin	Invitrogen	R25001
LiveBLAzer FRET B/G Loading Kit: Solution A, B, and C	Invitrogen	K1030
Recovery Cell Culture Freezing Medium	Invitrogen	12648
Fetal bovine serum (FBS), charcoal stripped	Invitrogen	12676-011
Chenodeoxycholic acid (CDCA)	Sigma	C9377
Black, clear-bottom, 1536-well assay plates	Greiner BioOne	789092-F
PinTool	Kalypsys	
BioRAPTR, Microfluidic Workstation	Beckmen	
EnVision plate reader	Perkin Elmer	

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Freezing Medium	-	-	-	100%
DMEM+Glutamax	90%	-	90%	-
Phenol Red free DMEM	-	98%	-	-
Dialyzed FBS	10%	-	10%	-
Charcoal-Stripped FBS	-	2%	-	-
Penicillin/Streptomycin	100U/mL;100 µg/mL	100U/mL;100 µg/mL	100U/mL;100 µg/mL	-
Sodium Pyruvate	-	1 mM	-	-
NEAA	0.1 mM	0.1 mM	0.1 mM	-
HEPES (pH 7.3)	25 mM	-	25 mM	-
Hygromycin	100 µg/ml	-	-	-
Zeocin	100 µg/ml	-	-	-

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube

1.2.2 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes.

1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.

1.2.4 Transfer the vial contents drop-wise into 14 mL of thaw medium in a sterile 15-mL conical tub.

1.2.5 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.

1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37 °C/5% CO2 incubator.

1.2.7 Switch to growth medium at first passage.

1.3. Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add 0.05% Trypsin/EDTA and swirl to coat the cell evenly.

1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.

1.3.3 Centrifuge cells at 900 rpm for 4 minutes and resuspend in growth medium.

1.3.4 Cell should be passage at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture and resuspend in assay medium.

2.2 Dispense 5000 cells/5µL/well into 1536-well tissue treated black, clear-bottom plates using a Multi-drop dispenser.

2.3 After the cells were incubated at 37°C for 5 hours, 23 nL of control or compounds dissolved in DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution.

2.4 Add 1 µL of agonist (CDCA) at 300 µM in assay medium to the column 1-2 and column 5-48. Add 1 µL of assay medium to the column 3-4.

2.5 Incubate the plates for 16 hours at 37°C.

2.6 Add 1 µL of 6X LiveBLAzer-FRET B/G (CCF4-AM) Substrate Mixture to each well using a BioRAPTR dispenser.

2.7 After two hours incubation at room temperature, measure fluorescence intensity at 460 and 530 nm emission and 405 nm excitation by an Envision detector. Data is expressed as the ratio of 460nm/530nm emissions.

Protocol Summary

FXR-bla cells were dispensed at 5000 cells/5uL/well in 1536-well black wall/clear bottom plates using a Multidrop Combi (Thermo Fisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 C and 5% CO2 for 5 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1 µL Chenodeoxycholic acid (50 uM, final concentration in the wells). The assay plates were incubated at 37C for 16 h. After 1 µL of LiveBLAzer B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN), the plates were incubated at room temperature for 2 h, and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 4µL/well of CellTiter-Glo reagent was added into the assay plates using a Bioraptr FRD. After 30 min incubation at room temperature, the luminescence intensity in the plates was measured using a ViewLux plate reader (PerkinElmer).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [3]. GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote FXR mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 farnesoid-X-receptor β-lactamase antagonist assay screened a library of diverse environmental compounds to probe for xenobiotic ligand-binding and potential to inhibit FXR-dependent transcription, monitored through *bla* reporter gene signal activation using a

mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 16 hour incubation of cells with test compounds and a small amount of agonist (50 µM chenodeoxycholic acid) a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for an additional hour, in the dark. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Farnesoid-X-receptor (FXR) is a ligand-activated nuclear receptor which regulates the expression of genes involved in bile acid homeostasis and has a role in the regulation of glucose and lipid metabolic pathways. FXR is primarily expressed in the liver, kidney, intestine and adrenal cortex, and regulates the expression of target genes by binding either as a monomer or as a heterodimer with the retinoid X receptor (RXR). Numerous studies have reported that FXR exerts protective function during cholestasis, diabetes, liver regeneration, and cancer. The FXR-RXR heterodimer, when bound to DNA, can act as transcriptional activators or inhibitors. FXR is activated by bile acids and the main endogenous ligand for FXR is chenodeoxycholic acid (CDCA). FXR reduces bile acid concentration in the liver by repressing genes involved in bile acid synthesis and regulates lipid metabolism.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.127
Neutral control median absolute deviation, by plate:	4.829
Positive control well median response value, by plate:	-100.38
Positive control well median absolute deviation, by plate:	6.094
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.67
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-20.67

Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	778.69
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-37.07

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

FXR ligand-binding and antagonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the FXR_BLA_Antagonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (Guggulsterone; 100% antagonist activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of Guggulsterone activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. FXR activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_IR1_CIS_up
ATG_FXR_TRANS_up
NVS_NR_hFXR_Agonist
NVS_NR_hFXR_Antagonist
OT_FXR_FXRSRC1_0480
OT_FXR_FXRSRC1_1440
Tox21_FXR_BLA_agonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM

Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.790
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [4].

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

ATCC, American Tissue Culture Collection
AIC, Akaike Information Criterion
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
FXR, Farnesoid X receptor
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
RXR, Retinoid X Receptor
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 October 2016

Date of Revisions:

Author of Revisions:

5. **Supporting Information (existing annotations):**

Assay Endpoint ID: 1124

Tox21_PPARd_BLA_agonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Peroxisome Proliferator-activated Receptor Delta (PPARd) Agonist qHTS Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors of the nuclear receptor superfamily with three distinct subtypes namely PPAR alpha, PPAR delta (PPARδ) (also called PPAR beta) and PPAR gamma. All these subtypes form heterodimers with Retinoid X receptor (RXR) to regulate transcription of various genes and have different physiological functions. Although the function of PPARδ is less well known, the recent identification of subtype specific synthetic ligands and the creation of animal models revealed its role in the regulation of cholesterol and lipid metabolism. To identify the compounds that activate PPARδ signaling, GeneBLAzer PPAR delta UAS-bla HEK293H cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under the control of an Upstream Activator Sequence (UAS) was used to screen the Tox21 compound library. This experimental system expresses a fusion protein of a peroxisome proliferator-activated receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds stimulates β-lactamase reporter gene expression. PPARδ activation by test compounds was assessed following 24-hour incubation in 1536-well microtiter plates. The cytotoxicity of the Tox21 compound library against the PPARδ-bla cell line was tested in parallel by measuring the cell viability using CellTiter-Glo assay (Promega, Madison, WI) in the same wells.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from xenobiotic PPAR delta gene expression.

Experimental System:

GeneBLAzer® PPAR delta 293T DA (Division-arrested) cells and PPAR delta-UAS-bla 293T cells contain a peroxisome proliferator-activated receptor delta (PPARδ) ligand-binding domain/Gal4 DNA-binding domain chimera, stably integrated into the CellSensor® UAS-bla 293T cell line. CellSensor® UAS-bla 293T contains a beta-lactamase reporter gene under control of a UAS response element stably integrated into 293T cells. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-PPAR delta (LBD) fusion protein, the protein binds to the UAS, resulting in expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM+Glutamax	Invitrogen	10569
Phenol Red free DMEM	Invitrogen	21063
Dialyzed FBS	Invitrogen	26400
Charcoal-Stripped FBS	Invitrogen	12676
Sodium Pyruvate	Invitrogen	11360
Penn-strep	Invitrogen	15140
NEAA	Invitrogen	11140
HEPES	Invitrogen	15630
HygromycinB	Invitrogen	10687
Zeocin	Invitrogen	R25001
Multidrop	Thermofisher	-
BiorapTR dispenser	Beckman Coulter	-
Envision plate reader	Perkin Elmer	-
LiveBLAzer B/G FRET substrate	Invitrogen	K1030

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Freezing Medium	-	-	-	100%
DMEM+Glutamax	90%	-	90%	-
Phenol Red free DMEM	-	98%	-	-
Dialyzed FBS	10%	-	10%	-
Charcoal-Stripped FBS	-	2%	-	-
Penn-strep	1%	1%	1%	-
Sodium Pyruvate	-	-	1 mM	-
NEAA	0.1 mM	-	0.1 mM	-
HEPES	25 mM	-	25 mM	-
Hygromycin	80 µg/ml	-	-	-
Zeocin	100 µg/ml	-	-	-

1.2. Thawing method

1.2.1 -Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube

1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 -Mix the entire content of the vial to 14 ml of pre-warmed medium and centrifuge to remove DMSO

1.2.4 -Discard the supernatant and transfer the precipitated cells to T175 flask using 30 ml thawing medium

1.3. Propagation method

1.3.1 - Detach the cells from the flask using TrypLExpress

1.3.2 -The cells are re-seeded in T-175 flask at 2.5-4 million

2. Assay Protocol

2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing

2.2 -Resuspend the pellet with assay medium followed by filtering through cell strainer and adjust the required cell density

2.3 -Plate the cells in black-clear bottom 1536 well plate at 3000/well/6µL through 8 tip Multidrop plate dispenser

2.4 -Incubate for 5hrs at 37°C / 99% Humidity / 5% CO2

2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.6 -Incubate for 17hrs at 37°C / 99% Humidity / 5% CO2

2.7 -Add 1 µL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)

2.8 -Incubate at room temperature for 1hrs in the dark

2.9 -Read the fluorescence intensity through Envision plate reader using Beta-Lactamaze protocol optimized for this cell type

2.10 -Add 3 µL of Cell Titer Glo and Incubate at room temperature for 0.5 hrs in dark for both agonist and antagonist mode

2.11 -Read on ViewLux protocol optimized for this cell type for agonist mode

PPARd-bla cells were dispensed at 3000 cells/6µL/well in 1536-well black wall/clear bottom plates using a Multidrop Combi (Thermo Fisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 °C and 5% CO2 for 5 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The assay plates were incubated at 37C for 17 h. After 1 µL of LiveBLAzer™ B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA), the plates were incubated at room temperature for 2 h, and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 3 µL/well of CellTiter-Glo reagent was added into the assay plates using a Bioraptr FRD. After 30 min incubation at room temperature, the luminescence intensity in the plates was measured using a ViewLux plate reader (PerkinElmer).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [3]. GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with

extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 peroxisome proliferator-activated receptor delta β-lactamase agonist assay screened a library of diverse environmental compounds to probe for xenobiotic ligand-binding and potential to induce PPAR delta-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 17-hour incubation of cells with test compounds a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for an additional 2 hours in the dark. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. Following CCF4 incubation and detection, 3µL of CellTiter-Glo reagent is added to each well, and incubated for 30 minutes before cytotoxicity readout is measured on a ViewLux microtiter plate reader. The test compounds were selected

based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical). 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Peroxisome proliferator-activated receptor delta is a ligand-activated nuclear receptor which is expressed ubiquitously and may have a role in regulating the differentiation of adipocytes, in keratinocyte differentiation and in the regulation of cholesterol and lipid metabolism [4, 5]. The PPARd_BLA_Agonist assay used Fluorescence Resonance Energy Transfer (FRET) substrate to generate a ratiometric reporter response to receptor ligand-binding to allow monitoring of PPARδ activity relative to a known receptor agonist. This assay is designed to help identify environmental compounds with a capacity for PPAR delta ligand-binding activity. The Tox21 PPARδ bla assays are qHTS format assays which measured the ability of a chemical to interact with PPARδ by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses PPARδ and a one-hybrid GAL4 system to quantify xenobiotic PPARδ agonism.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.144
Neutral control median absolute deviation, by plate:	4.064
Positive control well median response value, by plate:	99.74
Positive control well median absolute deviation, by plate:	7.63
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.64
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	24.57
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-693.78
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-26.69

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPAR delta ligand-binding and agonism as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the PPARd_BLA_Agonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (L-165,041; 100% activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of L-165,041 activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
ATG_PPARd_TRANS_up
Tox21_PPARd_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.916
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives,

food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [6].

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archives Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Schmuth, M., et al. (2004). J Invest Derm 122(4): 971-983. (PMID: 15102088)
[5] Seimandi, M., et al. (2005). Anal Biochem 344(1): 8-15. (PMID: 16038868)
[6] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

ATCC, American Tissue Culture Collection
AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
PPAR, Peroxisome Proliferator-Activated Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

15 July 2016

Date of Revisions:

Author of Revisions:

5. Supporting Information

Tox21_PPARd_BLA_antagonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Peroxisome Proliferator-activated Receptor Delta (PPARd) Antagonist qHTS Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors of the nuclear receptor superfamily with three distinct subtypes namely PPAR alpha, PPAR delta (PPARδ) (also called PPAR beta) and PPAR gamma. All these subtypes form heterodimers with Retinoid X receptor (RXR) to regulate transcription of various genes and have different physiological functions. Although the function of PPARδ is less well known, the recent identification of subtype specific synthetic ligands and the creation of animal models revealed its role in the regulation of cholesterol and lipid metabolism. To identify the compounds that inhibit PPAR-delta signaling, GeneBLAzer PPAR delta UAS-bla HEK293H cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under the control of an Upstream Activator Sequence (UAS) was used to screen the Tox21 compound library. This experimental system expresses a fusion protein of a peroxisome proliferator-activated receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds stimulates β-lactamase reporter gene expression. PPARδ inhibition by test compounds was assessed following 24 hour incubation in 1536-well microtiter plates. The cytotoxicity of the Tox21 compound library against the PPARδ-bla cell line was tested in parallel by measuring the cell viability using CellTiter-Glo assay (Promega, Madison, WI) in the same wells.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from xenobiotic PPAR delta gene expression.

Experimental System:

GeneBLAzer® PPAR delta 293T DA (Division-arrested) cells and PPAR delta-UAS-bla 293T cells contain a peroxisome proliferator-activated receptor delta (PPARδ) ligand-binding domain/Gal4 DNA-binding domain chimera, stably integrated into the CellSensor® UAS-bla 293T cell line. CellSensor® UAS-bla 293T contains a beta-lactamase reporter gene under control of a UAS response element stably integrated into 293T cells. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an antagonist binds to the LBD of the GAL4 (DBD)-PPAR delta (LBD) fusion protein, binding to the UAS is impeded, resulting in interfered expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 (Dohr et al. 1995). The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid (Bylund et al. 2004). HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM+Glutamax	Invitrogen	10569
Phenol Red free DMEM	Invitrogen	21063
Dialyzed FBS	Invitrogen	26400
Charcoal-Stripped FBS	Invitrogen	12676
Sodium Pyruvate	Invitrogen	11360
Penn-strep	Invitrogen	15140
NEAA	Invitrogen	11140
HEPES	Invitrogen	15630
HygromycinB	Invitrogen	10687
Zeocin	Invitrogen	R25001
Multidrop	Thermofisher	-
BiorapTR dispenser	Beckman Coulter	-
Envision plate reader	Perkin Elmer	-
LiveBLAzer B/G FRET substrate	Invitrogen	K1030

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Freezing Medium	-	-	-	100%
DMEM+Glutamax	90%	-	90%	undefined
Phenol Red free DMEM	-	98%	-	-
Dialyzed FBS	10%	-	10%	undefined
Charcoal-Stripped FBS	-	2%	-	-
Penn-strep	1%	1%	1%	-
Sodium Pyruvate	-	-	1 mM	-
NEAA	0.1 mM	-	0.1 mM	-
HEPES	25 mM	-	25 mM	-
Hygromycin	80 µg/ml	-	-	-
Zeocin	100 µg/ml	-	-	-

1.2. Thawing method

1.2.1 -Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube

1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 -Mix the entire content of the vial to 14 ml of pre-warmed medium and centrifuge to remove DMSO

1.2.4 -Discard the supernatant and transfer the precipitated cells to T175 flask using 30 ml thawing medium

1.3. Propagation method

1.3.1 - Detach the cells from the flask using TrypLExpress

1.3.2 -The cells are re-seeded in T-175 flask at 2.5-4 million

2. Assay Protocol

2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing

2.2 -Resuspend the pellet with assay medium followed by filtering through cell strainer and adjust the required cell density

2.3 -Plate the cells in black-clear bottom 1536 well plate at 3000/well/5µL through 8 tip Multidrop plate dispenser

2.4 -Incubate for 5hrs at 37°C / 99% Humidity / 5% CO2

2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.6 -Add 1 µL of buffer and 1µL of Agonist concentration to respective columns as per plate map for antagonist mode

2.7 -Incubate for 17hrs at 37°C / 99% Humidity / 5% CO2

2.8 -Add 1 µL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)

2.9 -Incubate at room temperature for 2hrs in the dark

2.10 -Read the fluorescence intensity through Envision plate reader using Beta-Lactamase protocol optimized for this cell type

2.11 -Add 3 µL of Cell Titer Glo and Incubate at room temperature for 0.5 hrs in dark for both agonist and antagonist mode

2.12 -Read on ViewLux protocol optimized for this cell type for antagonist mode

PPARd-bla cells were dispensed at 3000 cells/5uL/well in 1536-well black wall/clear bottom plates using a Multidrop Combi (Thermo Fisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 C and 5% CO2 for 5 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1ul L-165,041 (300 nM, final concentration in the wells). The assay plates were incubated at 37 C for 17 h, and then 1 uL of LiveBLAzerTM B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The assay plates were incubated at room temperature for 2 h and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 3 ul/well of CellTiter-Glo reagent was added into the assay plates using a Bioraptr FRD. After 30 min incubation at room temperature, the luminescence intensity in the plates was measured using a ViewLux plate reader (PerkinElmer).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) (Michael et al. 2008). GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

2. Assay Component Descriptions

Assay Objectives:

The Tox21 peroxisome proliferator-activated receptor delta β-lactamase antagonist assay screened a library of diverse environmental compounds to probe for xenobiotic ligand-binding and potential to suppress PPARδ-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. Each well contained 0.3 µM L-165,041 to stimulate receptor activity and MK886 (a leukotriene inhibitor) served as a positive control. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 17 hour incubation of cells with test compounds a membrane-permeable FRET-based substrate

CCF4-AM is introduced and incubated for an additional hour. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader and CellTiter-Glo assay reagent (Promega) is also incubated with test system for 30 minutes before readout to detect cell viability.

The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical). 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Peroxisome proliferator-activated receptor delta is a ligand-activated nuclear receptor which is expressed ubiquitously and may have a role in regulating the differentiation of adipocytes, in keratinocyte differentiation and in the regulation of cholesterol and lipid metabolism (Schmuth et al. 2004, Seimandi et al. 2005). The PPARδ_BLA_Antagonist assay used Fluorescence Resonance Energy Transfer (FRET) substrate to generate a ratiometric reporter response to receptor ligand-binding to allow monitoring of PPARδ activity relative to a known receptor antagonist. This assay is designed to help identify environmental compounds with a capacity for PPAR delta interfering activity. The Tox21 PPARδ bla assays are qHTS format assays which measured the ability of a chemical to interact with PPARδ by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses PPARδ and a one-hybrid GAL4 system to quantify xenobiotic PPARδ agonism.

Method Development Reference:

Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A., Zheng, W., & Austin, C. P. (2006). "Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries". Proc Natl Acad Sci 103(31), 11473-11478. (PMID: 16864780)

Xia, M., Huang, R., Sun, Y., Semenza, G. L., Aldred, S. F., Witt, K. L., Inglese, J., Tice, R. R., & Austin, C. P. (2009). "Identification of chemical compounds that induce HIF-1alpha activity". Toxicol Sci 112(1), 153-163. (PMID: 19502547)

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.1035
Neutral control median absolute deviation, by plate:	1.4826
Positive control well median response value, by plate:	133.452
Positive control well median absolute deviation, by plate:	11.6836
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z’ (median across all plates, using positive control wells):	0.68
Z’ (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	90.3
Signal-to-noise (median across all plates, using negative control wells):	NA

Signal-to-background (median across all plates, using positive control wells):	14.2
Signal-to-background (median across all plates, using positive control wells):	NA
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPAR delta antagonism and ligand interference and as monitored by FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the PPARd_BLA_antagonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (MK886; 100% activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of MK886 activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR delta interence was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.302
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals (Richard et al. 2016).

4. Assay Documentation

4.1. References

4.2. Abbreviations and Definitions

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard and provide a gauge of potency;

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Supporting Information (existing annotations):

Tox21_PPARg_BLA_Agonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Peroxisome Proliferator-activated Receptor Gamma (PPARg) Agonist qHTS Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors of the nuclear receptor superfamily with three distinct subtypes namely PPAR alpha, PPAR delta (also called PPAR beta) and PPAR gamma. All these subtypes heterodimerize with Retinoid X receptor (RXR) and these heterodimers regulate transcription of various genes. The PPARγ receptor is involved in the regulation of glucose and lipid metabolism. To identify the compounds that activate PPARγ signaling, GeneBLAzer® PPAR gamma UAS-bla HEK293H cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under control of an upstream activator sequence (UAS) stably integrated into HEK293H cells was used to screen the Tox21 compound library. This experimental system expresses a fusion protein of a peroxisome proliferator-activated receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds stimulates β-lactamase reporter gene expression. PPARγ activation by test compounds was assessed following 24 hour incubation in 1536-well microtiter plates.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from xenobiotic PPAR delta gene expression.

Experimental System:

GeneBLAzer® PPARγ 293H DA (Division-arrested) cells and PPAR gamma-UAS-bla 293H cells contain a peroxisome proliferator-activated receptor gamma (PPARγ) ligand-binding domain/Gal4 DNA-binding domain chimera, stably integrated into the CellSensor® UAS-bla 293H cell line. CellSensor® UAS-bla 293H contains a beta-lactamase reporter gene under control of a UAS response element stably integrated into 293H cells. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-PPARγ (LBD) fusion protein, the protein binds to the UAS, resulting in expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 [1]. The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid [2]. HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM+Glutamax	Invitrogen	11965
Phenol Red free DMEM	Invitrogen	21063
Dialyzed FBS	Invitrogen	26400
Charcoal-Stripped FBS	Invitrogen	12676-029
Sodium Pyruvate	Invitrogen	11360
Penn-strep	Invitrogen	15140
NEAA	Invitrogen	11140
HEPES	Invitrogen	15630
Hygromycin	Invitrogen	10687-010
Geneticin	Invitrogen	10131-027
Multidrop	Thermofisher	-
BiorapTR dispenser	Beckman Coulter	-
Envision plate reader	Perkin Elmer	-
LiveBLAzer B/G FRET substrate	Invitrogen	K1030

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Freezing Medium	-	-	-	100%
DMEM+Glutamax	90%	-	90%	-
Phenol Red free DMEM	-	99%	-	-
Dialyzed FBS	10%	-	10%	-
Charcoal-Stripped FBS	-	1%	-	-
Penn-strep	1%	1%	1%	-
Sodium Pyruvate	1 mM	-	1 mM	-
NEAA	0.1 mM	-	0.1 mM	-
HEPES	25 mM	-	25 mM	-
Hygromycin	100 ug/ml	-	-	-
Geneticin	500 ug/ml	-	-	-

1.2. Thawing method

1.2.1 -Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube

1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 -Mix the entire content of the vial to 14 ml of pre-warmed medium and centrifuge to remove DMSO

1.2.4 -Discard the supernatant and transfer the precipitated cells to T175 flask using 30 ml thawing medium

1.3. Propagation method

1.3.1 - Detach the cells from the flask using TrypLExpress

1.3.2 -The cells are re-seeded in T-175 flask at 3-4 million

2. Assay Protocol

2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing

2.2 -Resuspend the pellet with assay medium followed by filtering through cell strainer and adjust the required cell density

2.3 -Plate the cells in black-clear bottom 1536 well plate at 3000/well/6µL through 8 tip Multidrop plate dispenser

2.4 -Incubate for 5hrs at 37°C / 99% Humidity / 5% CO2

2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.6 -Incubate for 17hrs at 37°C / 99% Humidity / 5% CO2

2.7 -Add 1 µL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)

2.8 -Incubate at room temperature for 1hrs in the dark

2.9 -Read the fluorescence intensity through Envision plate reader using Beta-Lactamaze protocol optimized for this cell type

PPARγ-bla cells were dispensed at 3000 cells/6uL/well in 1536-well black wall/clear bottom plates using a Multidrop Combi (Thermo Fisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37°C and 5% CO2 for 5 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA). The assay plates were incubated at 37C for 17 h. After 1 uL of LiveBLAzerTM B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA), the plates were incubated at room temperature for 1 h, and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [3]. GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program

National Center for Advancing Translational Sciences [NCATS]

NIH Chemical Genomics Center [NCGC]

U.S. Environmental Protection Agency [EPA]

National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 peroxisome proliferator-activated receptor gamma β-lactamase agonist assay screened a library of diverse environmental compounds to probe for xenobiotic ligand-binding and potential to induce PPARγ-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 17 hour incubation of cells with test compounds a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for an additional hour, in the dark. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. The test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known ER agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor which regulates the expression of genes involved in fatty acid-oxidation and is a major regulator of

energy homeostasis. PPARγ is primarily expressed in adipose tissue, macrophages and in the colon where it controls adipocyte differentiation, lipid storage and inflammatory responses. PPARγ agonists, the thiazolidinediones (TZDs), improve insulin sensitivity, lower glucose levels, and lower plasma triglycerides and free fatty acid (FFA) levels by enhancing their uptake into adipocytes. The PPARg_BLA_Agonist assay used Fluorescence Resonance Energy Transfer (FRET) substrate to generate a ratiometric reporter response to receptor ligand-binding to allow monitoring of PPARγ activity relative to a known receptor agonist. This assay is designed to help identify environmental compounds with a capacity for PPARγ ligand-binding activity. The Tox21 PPARγ bla assays are qHTS format assays which measured the ability of a chemical to interact with PPARγ by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses PPARγ and a one-hybrid GAL4 system to quantify xenobiotic PPARγ agonism**.**

Method Development Reference:

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.093
Neutral control median absolute deviation, by plate:	3.487
Positive control well median response value, by plate:	99.805
Positive control well median absolute deviation, by plate:	6.461
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.7
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	14
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	28.66
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-953.1
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-34.37

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPAR gamma ligand-binding and agonism; measured by monitoring FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the PPARg_BLA_Agonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (rosiglitazone; 100% activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of rosiglitazone activity, and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model

which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR gamma activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
ATG_PPARg_TRANS_up
NVS_NR_hPPARg
OT_PPARg_PPARgSRC1_0480
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_antagonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.084
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds

recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [11].

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)
[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)
[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)
[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

ATCC, American Tissue Culture Collection
AIC, Akaike Information Criterion
AOP, Adverse Outcome Pathway
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
LBD, Ligand-Binding Domain
MIE, Molecular Initiating Event
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
PPAR, Peroxisome Proliferator-Activated Receptors
qHTS, Quantitative High-Throughput Screening
UAS, Upstream Activator Sequence
TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

12 September 2016

Date of Revisions:

Author of Revisions:

5. Supporting Information

Tox21_PPARg_BLA_antagonist_ratio

Assay Name: Tox21 Beta-lactamase HEK293 Peroxisome Proliferator-activated Receptor Gamma (PPARg) Antagonist qHTS Assay

1. Assay Descriptions

1.1. Overview

Assay Summary:

The peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors of the nuclear receptor superfamily with three distinct subtypes namely PPAR alpha, PPAR delta (also called PPAR beta) and PPAR gamma (PPARγ). All these subtypes heterodimerize with Retinoid X receptor (RXR) and these heterodimers regulate transcription of various genes. PPARγ receptor (glitazone receptor) is involved in the regulation of glucose and lipid metabolism. To identify the compounds that inhibit PPAR-gamma signaling, GeneBLAzer PPAR gamma UAS-bla HEK293H cell line (Invitrogen, Carlsbad, CA, USA) containing a beta-lactamase reporter gene under control of an Upstream Activator Sequence (UAS) was used to screen the Tox21 compound library. This experimental system expresses a fusion protein of a peroxisome proliferator-activated receptor ligand-binding domain coupled to GAL4 DNA-binding domain which when activated by xenobiotic compounds modulates β-lactamase reporter gene expression. PPARγ interactions with tested compounds was assessed following 24 hour incubation in 1536-well microtiter plates. To detect loss of signal due to compound cytotoxicity, a CellTiter-Glo fluorescence assay to measure ATP production was run concurrently in all wells using tetraoctylammonium bromide as a positive control for cell death.

1.2. Assay Definition

Assay Throughput:

Stably transfected HEK293T cells are aliquoted into 1536-well microtiter plates and incubated with test compounds for 24 hours prior to monitoring fluorescence emission resulting from PPAR delta gene expression.

Experimental System:

GeneBLAzer® PPARγ 293H DA (Division-arrested) cells and PPAR gamma-UAS-bla 293H cells contain a peroxisome proliferator-activated receptor gamma (PPARγ) ligand-binding domain/Gal4 DNA-binding domain chimera, stably integrated into the CellSensor® UAS-bla 293H cell line. CellSensor® UAS-bla 293H contains a beta-lactamase reporter gene under control of a UAS response element stably integrated into 293H cells. These cells stably express a β-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an antagonist binds to the LBD of the GAL4 (DBD)-PPARγ (LBD) fusion protein, binding to the UAS is impeded, resulting in interfered expression of β-lactamase.

The HEK-293 cell line is a human embryonic kidney cell line (of unknown parentage) transformed with sheared adenovirus 5 DNA by Frank Graham in 1973 (Dohr et al. 1995). The transformation incorporated approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells, and subsequent cytogenetic characterization established that the 293 line is pseudotriploid (Bylund et al. 2004). HEK293 cells are popular for their ease of growth and transfection cells, and are frequently used to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

Xenobiotic Biotransformation Potential:

The intrinsic production of Phase I/II enzymes has not been well characterized in this cell line and metabolic activation/detoxification of test compounds is potentially limited.

Basic Procedure:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM+Glutamax	Invitrogen	11965
Phenol Red free DMEM	Invitrogen	21063
Dialyzed FBS	Invitrogen	26400
Charcoal-Stripped FBS	Invitrogen	12676-029
Sodium Pyruvate	Invitrogen	11360
Penn-strep	Invitrogen	15140
NEAA	Invitrogen	11140
HEPES	Invitrogen	15630
Hygromycin	Invitrogen	10687-010
Geneticin	Invitrogen	10131-027
Multidrop	Thermofisher	-
BiorapTR dispenser	Beckman Coulter	-
Envision plate reader	Perkin Elmer	-
LiveBLAzer B/G FRET substrate	Invitrogen	K1030

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Freezing Medium	-	-	-	100%
DMEM+Glutamax	90%	-	90%	-
Phenol Red free DMEM	-	99%	-	-
Dialyzed FBS	10%	-	10%	-
Charcoal-Stripped FBS	-	1%	-	-
Penn-strep	1%	1%	1%	-
Sodium Pyruvate	1 mM	-	1 mM	-
NEAA	0.1 mM	-	0.1 mM	-
HEPES	25 mM	-	25 mM	-
Hygromycin	100 ug/ml	-	-	-
Geneticin	500 ug/ml	-	-	-

1.2. Thawing method

1.2.1 -Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube

1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 -Mix the entire content of the vial to 14 ml of pre-warmed medium and centrifuge to remove DMSO

1.2.4 -Discard the supernatant and transfer the precipitated cells to T175 flask using 30 ml thawing medium

1.3. Propagation method

1.3.1 - Detach the cells from the flask using TrypLExpress

1.3.2 -The cells are re-seeded in T-175 flask at 3-4 million

2. Assay Protocol

2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing

2.2 -Resuspend the pellet with assay medium followed by filtering through cell strainer and adjust the required cell density

2.3 -Plate the cells in black-clear bottom 1536 well plate at 3000/well/5µL through 8 tip Multidrop plate dispenser

2.4 -Incubate for 5hrs at 37°C / 99% Humidity / 5% CO2

2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.6 -Add 1 uL of buffer and 1uL of Agonist concentration to respective columns as per plate map for antagonist mode

2.7 -Incubate for 17hrs at 37°C / 99% Humidity / 5% CO2

2.8 -Add 1 µL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)

2.9 -Incubate at room temperature for 1hrs in the dark

2.10 -Read the fluorescence intensity through Envision plate reader using Beta-Lactamaze protocol optimized for this cell type

2.11 -Add 3 uL of Cell Titer Glo and Incubate at room temperature for 0.5 hrs in dark

2.12 -Read on ViewLux protocol optimized for this cell type for antagonist mode

PPARg-bla cells were dispensed at 3000 cells/5uL/well in 1536-well black wall/clear bottom plates using a Multidrop Combi (Thermo Fisher Scientific, Waltham, MA) dispenser. After the assay plates were incubated at 37 C and 5% CO2 for 5 h, 23 nL of compounds dissolved in DMSO, positive controls or DMSO only was transferred to the assay plate by a Pintool station (Kalypsys, San Diego, CA), followed by addition of 1ul Rosiglitazone (50 nM, final concentration in the wells). The assay plates were incubated at 37°C for 17 h, and then 1 uL of LiveBLAzerTM B/G FRET substrate was added using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The assay plates were incubated at room temperature for 1 h and fluorescence intensity was measured by an Envision plate reader (PerkinElmer, Shelton, CT). For cell viability readout that measures cytotoxicity, 4 ul/well of CellTiter-Glo reagent was added into the assay plates using a Flying Reagent Dispenser. After 30 min incubation at room temperature, the luminescence intensity in the plates was measured using a ViewLux plate reader (PerkinElmer).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) (Michael et al. 2008). GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote peroxisome proliferator receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with

extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., & Austin, C. P. (2011). "Chemical genomics profiling of environmental chemical modulation of human nuclear receptors". Environ Health Perspect 119(8), 1142-1148. (PMID: 21543282)

Huang, R., Xia, M., Sakamuru, S., Zhao, J., Shahane, S. A., Attene-Ramos, M., Zhao, T., Austin, C. P., & Simeonov, A. (2016). "Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization". Nature Communications 7, 10425. (PMID: 26811972)

Teng, C., Goodwin, B., Shockley, K., Xia, M., Huang, R., Norris, J., Merrick, B. A., Jetten, A. M., Austin, C. P., & Tice, R. R. (2013). "Bisphenol A affects androgen receptor function via multiple mechanisms". Chem-Biol Interact 203(3), 556-564. (PMID: 23562765)

Method Updates / Confirmatory Studies:

None reported

2. Assay Component Descriptions

Assay Objectives:

The Tox21 peroxisome proliferator-activated receptor gamma β-lactamase antagonist assay screened a library of diverse environmental compounds to probe for xenobiotic ligand-interference and potential to suppress PPARγ-dependent transcription, monitored through *bla* reporter gene signal activation using a mammalian one-hybrid GAL4 system. The assay is run in triplicate on 1536-well microplates. HEK293T cells are plated the day of the assay and following 17 hour incubation of cells with test compounds a membrane-permeable FRET-based substrate CCF4-AM is introduced and incubated for an additional hour. Once in the cell, cytoplasmic esterases trap the negatively charged CCF4 substrate in the cytosol and *bla* expression is quantified by measuring the ratio of blue (460nm, product) to green (530nm, substrate) fluorescence. Fluorescence signals are monitored using an Envision plate reader. The test compounds were selected based on various

criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical). 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at potentially interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor which regulates the expression of genes involved in fatty acid-oxidation and is a major regulator of energy homeostasis. PPARγ is primarily expressed in adipose tissue, macrophages and in the colon where it controls adipocyte differentiation, lipid storage and inflammatory responses. PPARγ agonists, the thiazolidinediones (TZDs), improve insulin sensitivity, lower glucose levels, and lower plasma triglycerides and free fatty acid (FFA) levels by enhancing their uptake into adipocytes. The PPARg_BLA_Antagonist assay used Fluorescence Resonance Energy Transfer (FRET) substrate to generate a ratiometric reporter response to receptor ligand-binding to allow monitoring of PPARγ activity relative to a known receptor antagonist. This assay is designed to help identify environmental compounds with a capacity for PPARγ ligand-binding interference. The Tox21 PPARγ bla assays are qHTS format assays which measured the ability of a chemical to inhibit PPARγ by monitoring modulation of fluorescence reporter gene signals. This assay utilized a human embryonic kidney cell line (HEK293T) which expresses PPARγ and a one-hybrid GAL4 system to quantify xenobiotic PPARγ antagonism.

Method Development Reference:

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.154
Neutral control median absolute deviation, by plate:	10.059
Positive control well median response value, by plate:	-100.23
Positive control well median absolute deviation, by plate:	5.506
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.53
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	-9.95
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	583.55
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-61.24

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

PPAR gamma ligand-binding and antagonism; measured by monitoring FRET emission resulting from GAL4/β-lactamase gene expression.

Analytical Elements:

BLA expression in the PPARg_BLA_Antagonist assay is quantified by measuring the ratio of product (blue; 460 nm) to substrate (green; 530 nm) fluorescence. Raw plate readouts were normalized relative to positive controls (GW9662; 100% PPARγ antagonist activity) and negative controls (DMSO; baseline activity) and then subjected to a NCGC in-house pattern correction algorithm (developed using DMSO-only plates run at the beginning and end of the compound plate stack). Data was analyzed as percentage of GW9662 activity and concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. PPAR gamma activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_PPRE_CIS_up
ATG_PPARg_TRANS_up
NVS_NR_hPPARg
OT_PPARg_PPARgSRC1_0480
OT_PPARg_PPARgSRC1_1440
Tox21_PPARg_BLA_agonist_ratio

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	80 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.000
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	35.98

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives,

food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals (Richard et al. 2016).

4. Assay Documentation

4.1. References

[1] Dohr, O., et al. (1995). Archiv Biochem Biophys 321(2): 405-412. (PMID: 7646066)

[2] Bylund, L., et al. (2004). Cytogenet Genome Res 106(1): 28-32. (PMID: 15218237)

[3] Michael, S., et al. (2008). Assay Drug Dev Technol 6(5): 637-657. (PMID: 19035846)

[4] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

ATCC, American Tissue Culture Collection

AIC, Akaike Information Criterion

FBS, Fetal Bovine Serum

FRD, Flying Reagent Dispenser

ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

LBD, Ligand-Binding Domain

NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods

NR, Nuclear Receptors

PPAR, Peroxisome Proliferator-Activated Receptors

qHTS, Quantitative High-Throughput Screening

UAS, Upstream Activator Sequence

TF, Transcription Factor

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-01)

Research Triangle Park, NC 27711

919-541-4219

Date of Assay Document Creation:

2017

Date of Revisions:

Author of Revisions:

5. Potential Regulatory Applications

Context of use:

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation
Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency;
Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. **Supporting Information (existing annotations):**

Tox21_TR_LUC_GH3_Agonist

Assay Name: Tox21 GH3 Cell-Based qHTS Luciferase Assay to Identify Small Molecule Agonists of the Thyroid Receptor (TR) Signaling Pathway by Monitoring Thyroid Response Element Activation

1. Assay Descriptions

1.1. Overview

Assay Summary:

Thyroid receptor (TR), a nuclear hormone receptor, plays an important role in development, proliferation, differentiation, metabolism, brain function, and cardiovascular system. TR-interacting compounds have been shown to disrupt thyroid homeostasis [1]. To profile the Tox21 compound library's potential to mimic TH and disrupt signaling pathways, a cell-based GH3-TRE-Luc assay was used to measure the activation of TR following xenobiotic exposures. Activity was measured in GH3 (rat pituitary tumor) cells stably expressing a TR activity sensor consisting of two TR response elements and a luciferase reporter gene in 1536-well plates following 24-hour incubation with test chemicals. Increased luciferase activity indicates elevated levels of TR transactivation.

1.2. Assay Definition

Assay Throughput:

GH3.TRE-Luc cells are aliquoted into 1536-well microtiter plates (1500 cells/5 µL/well) and incubated for 4 hours prior to 24-hour exposure to test compounds and monitoring of increased luminescence resulting from xenobiotic-induced TR gene expression.

Experimental System:

GH3 cell line was derived from rat pituitary tumor cells and has been routinely employed for studying effects of TH disruption using the T-screen assay [2, 3] and is reported to retain unique characteristics of the original differentiated tissue such as production of growth hormone and prolactin [3-5]. Moreover, this cell line endogenously expresses both TH receptor isoforms (α and β) in very high amounts and they respond to physiological concentrations of TH by proliferating [6]. The GH3.TRE-Luc cell line, developed in the laboratory of Dr. Albertinka J. Murk (Wageningen University) is derived from GH3 cells and stably expresses a modified firefly luciferase reporter gene under the regulation of a TR activity sensor consisting of a pair of thyroid hormone response elements (TREs). [7].

Xenobiotic Biotransformation Potential:

GH3 cells display an increased level of cell proliferation and growth hormone secretion in response to physiologic levels of thyroid hormones. TRE-LUC cells are activated by the thyroid hormone triiodothyronine (T3) and all trans retinoic acid but not RAR or LXR specific ligands. [7] CYP1A1 and 1B1 (but not 1A2) have been shown to be inducible by PCBs in GH3 cells [8].

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM:F12	Invitrogen	Gibco, 10565
Fetal Bovine Serum	Hyclone	Hyclone, Sh30071.03
Pen/Strep	Invitrogen	Invitrogen, 15140
Insulin	Sigma	Sigma, I6634

Ethanolamine	Sigma	Sigma, E0135
Sodium Selenite	Sigma	Sigma, S5261
Human Apotranferin	Sigma	Sigma, T2036
Bovine Serum Albumin	Sigma	Sigma, A9647
TrypLE Express	Invitrogen	Invitrogen, 12605
PBS w/o Calcium and Magnesium	Invitrogen	Invitrogen, 14190
Recovery Cell Culture Medium	Invitrogen	Invitrogen, 12648
Centrifuge	Sorvall Legend XTR	Thermo Fisher Science 75004520
Bioraptr Microfluidic Workstation	Beckmen	-
Pintool	Kalypsys	-
White, TC, Sterile 1536-Well Assay Plates	Greiner Bio-One	Greiner, 789173-F
Viewlux Plate Reader	Perkinelmer	-
T3 (Agonist control compound)	Calbiochem	Calbiochem, 642511
DMSO	AMRESCO	Kd Medical, RGE-3070
One-Glo	Promega	Promega, E6120

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Culture Medium	-	-	-	100%
DMEM: F12	90%	100%	90%	-
Fetal Bovine Serum	10%	-	10%	-
Pen/Strep	100U/mL-100µg/mL	-	100U/mL-100µg/mL	-
Insulin	-	10µg/mL	-	-
Ethanolamine	-	10µM	-	-
Sodium Selenite		10ng/mL	-	-
Human apo-Transferrin	-	10µg/mL	-	-
Bovine Serum Albumin	-	500µg/mL	-	-

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a T75 flask

1.2.2 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.

1.2.4 Transfer the vial contents drop-wise into 10 mL of Thaw Medium in a sterile 15-mL conical tube

1.2.5 Centrifuge cells at 1000 rpm for 4 minutes

1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37°C/5% CO2 incubator

1.2.7 Switch to growth medium at first passage.

1.3. Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add TrypLE Express (3 mL for a T75 flask and 5 mL for a T175 flask and 7.5 mL for T225 flask) and swirl to coat the cell evenly.

1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.

1.3.3 Centrifuge cells at 1000 rpm for 4 minutes and resuspend in Growth Medium.

1.3.4 Cell should be passage or fed at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture in Growth Medium and resuspend in assay medium

2.2 Dispense 1500 cells/5 µL/well into 1536-well tissue treated white solid plates using a BioRAPTR dispenser.

2.3 After the cells were incubated at 37°C for 4 hours, 23 nL of compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool

2.4 Incubate the plates for 24 hours at 37°C.

2.5 Add 5 µL of One-Glo to each well using a BioRAPTR dispenser and incubate the plate at room temperature for 30 mins.

2.6 Measure luminescence using Viewlux

GH3.TRE-Luc cells were dispensed at 1500 cells/5 µL/well into 1536-well white solid bottom plates using a Multidrop Combi dispenser (Thermo Fisher Scientific Inc., Waltham, MA). After the assay plates were incubated at 37°C for 4 h, 23 nL of library compound or controls (T3 or DMSO) was transferred to the assay plates by a pintool station (Kalypsys, San Diego, CA). The assay plates were incubated at 37°C for 24 hours. 5 µL of ONE-Glo Luciferase Assay reagent (Promega, Madison, WI) was added to each well using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA), followed by 30 min incubation at room temperature. The luminescence intensity of plates was recorded by a ViewLux plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [3].

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote thyroid receptor mediated gene expression, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program

National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2011

Assay Publication:

Freitas, J., Cano, P., Craig-Veit, C., Goodson, M. L., Furlow, J. D., & Murk, A. J. (2011). "Detection of thyroid hormone receptor disruptors by a novel stable in vitro reporter gene assay". Toxicol In Vitro 25(1), 257-266.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 thyroid receptor luciferase GH3 agonist assay screened a large library of diverse environmental compounds to probe for xenobiotic activity and potential to induce thyroid-dependent transcription, monitored through luciferase reporter gene signal activation using a TR-luciferase reporter gene construct. The assay is run in triplicate on a 1536-well microplate and bioluminescence was measured following 24-hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in agonist mode using luciferase detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

Thyroid hormone signaling is essential for normal brain development both before and after birth and has profound effects on cellular metabolism in almost all organs. One potential mechanism by which endocrine disrupting chemicals may produce toxic effects is by interfering with the ability of thyroid hormones (T3, triiodothyronine and T4, thyroxine) to direct normal development and metabolism. Thyroid hormone interfering compounds can result in neurological disorders by interfering with normal developmental processes. Thyroid hormones also have important roles in the initiation and proliferation of central nervous system and cardiovascular tissues. An important component of an endocrine disruptor screening program should be the inclusion of assays designed to screen TH disrupting compounds. The GH3_TRE_LUC cell line is a TR responsive system which is a sensitive and reliable method to evaluate the potential for xenobiotic compounds to act

as ligands to the thyroid hormone receptor. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of TR binding and interference with endogenous thyroid signaling by monitoring the increase in luminescent signals relative to a thyroid hormone (20mM T3) as a positive control, and indicator of receptor activation.

Method Development Reference:

Gutleb, A. C., Meerts, I. A., Bergsma, J. H., Schriks, M., & Murk, A. J. (2005). "T-Screen as a tool to identify thyroid hormone receptor active compounds". Environ Toxicol Pharmacol 19(2), 231-238. (PMID: 21783481)

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.040
Neutral control median absolute deviation, by plate:	1.602
Positive control well median response value, by plate:	99.59
Positive control well median absolute deviation, by plate:	12.64
Negative control well median, by plate:	NA
Negative control well median absolute deviation value, by plate:	NA
Z' (median across all plates, using positive control wells):	0.57
Z' (median across all plates, using negative control wells):	NA
SSMD (median across all plates, using positive control wells):	8
SSMD (median across all plates, using negative control wells):	NA
Signal-to-noise (median across all plates, using positive control wells):	62.07
Signal-to-noise (median across all plates, using negative control wells):	NA
Signal-to-background (median across all plates, using positive control wells):	-2484.75
Signal-to-background (median across all plates, using negative control wells):	NA
CV (median across all plates):	-39.06

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Thyroid receptor ligand-binding and agonism, measured by monitoring increased luminescence resulting from thyroid response element-driven expression of luciferase.

Analytical Elements:

The Tox21_TR_LUC_GH3_Agonist assay was monitored for increased luminescence (gain-of-signal) relative to 20mM T3 (positive control) signal, using DMSO (negative control) as a baseline for chemical-TR activity, and response was reported as a percent of positive control activity. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Thyroid receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were

determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRa1_TRANS_up
ATG_THRb_TRANS2_up
LTEA_HepaRG_THRSP_up
LTEA_HepaRG_THRSP_dn
NVS_NR_hTRa_Antagonist
Tox21_TR_LUC_GH3_Antagonist

3.2. Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	90 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.643
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [9].

4. Assay Documentation

4.1. References

[1] Crofton, K. M. (2008). Int J Androl 31(2): 209-223.
[2] Gutleb, A. C., et al. (2005). Environ Toxicol Pharmacol 19(2): 231-238. (PMID: 21783481)
[3] Hohenwarter, O., et al. (1996). Anal Biochem 234(1): 56-59.

[4] Ghisari, M. and E. C. Bonefeld-Jorgensen (2005). Mol Cell Endocrinol 244(1): 31-41. (PMID: 16221524)
[5] Samuels, H., et al. (1988). J Clin Invest 81(4): 957.
[6] Freitas, J., et al. (2011). Toxicol In Vitro 25(1): 257-266.
[7] Freitas, J., et al. (2014). Current Chem Genom Transl Med 8: 36.
[8] Gauger, K. J., et al. (2007). Environ Health Perspect: 1623-1630.
[9] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods
NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
T3, Triiodothyronine
T4, Thyroxine
TF, Transcription Factor
TH, Thyroid Hormone
TR, Thyroid Receptor
TRE, Thyroid Hormone Response Elements

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

3 August 2016

Date of Revisions:

Author of Revisions:

5. Supporting Information

Assay Endpoint ID: 804

Tox21_TR_LUC_GH3_Antagonist

Assay Name: Tox21 GH3 Cell-Based qHTS Luciferase Assay to Identify Small Molecule Antagonists of the Thyroid Receptor (TR) Signaling Pathway by Monitoring Thyroid Response Element Inhibition

1. Assay Descriptions

1.1. Overview

Assay Summary:

Thyroid receptor (TR), a nuclear hormone receptor, plays an important role in development, proliferation, differentiation, metabolism, brain function, and cardiovascular system. TR-interacting compounds have been shown to disrupt thyroid homeostasis [1]. To profile the Tox21 compound library's potential to interfere in TR signaling pathways, a cell-based GH3-TRE-Luc assay was used to measure the inhibition of TR following xenobiotic exposures. Activity was measured in GH3 (rat pituitary tumor) cells stably expressing a TR activity sensor consisting of two TR response elements and a luciferase reporter gene in 1536-well plates following 24-hour incubation with test chemicals. Increased luciferase activity indicates elevated levels of TR transactivation, and to detect TR antagonism, this assay is designed to monitor for loss-of-signal against thyroid hormone (T3) agonists. The cytotoxicity of the Tox21 compound library against the GH3.TRE-Luc cell line was tested in parallel by measuring the cell viability using CellTiter-Fluor assay (Promega, Madison, WI) in the same wells, using tetraoctylammonium bromide as a positive control for cytotoxicity.

1.2. Assay Definition

Assay Throughput:

GH3.TRE-Luc cells are aliquoted into 1536-well microtiter plates (1500 cells/5 µL/well) and incubated for 4 hours prior to 24-hour exposure to test compounds in the presence of T3 (agonist). Antagonistic activity is monitoring by measuring decreased luminescence resulting from xenobiotic-repression of TR gene expression.

Experimental System:

GH3 cell line was derived from rat pituitary tumor cells and has been routinely employed for studying effects of TH disruption using the T-screen assay [2, 3] and is reported to retain unique characteristics of the original differentiated tissue such as production of growth hormone and prolactin [3-5]. Moreover, this cell line endogenously expresses both TH receptor isoforms (α and β) in very high amounts and they respond to physiological concentrations of TH by proliferating [6]. The GH3.TRE-Luc cell line, developed in the laboratory of Dr. Albertinka J. Murk (Wageningen University) is derived from GH3 cells and stably expresses a modified firefly luciferase reporter gene under the regulation of a TR activity sensor consisting of a pair of thyroid hormone response elements (TREs). [7].

Xenobiotic Biotransformation Potential:

GH3 cells display an increased level of cell proliferation and growth hormone secretion in response to physiologic levels of thyroid hormones. TRE-LUC cells are activated by the thyroid hormone triiodothyronine (T3) and all trans retinoic acid but not RAR or LXR specific ligands. [2] CYP1A1 and 1B1 (but not 1A2) have been shown to be inducible by PCBs in GH3 cells [3].

Basic Procedure:

Materials

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM:F12	Invitrogen	Gibco, 10565

Fetal Bovine Serum	Hyclone	Hyclone, Sh30071.03
Pen/Strep	Invitrogen	Invitrogen, 15140
Insulin	Sigma	Sigma, I6634
Ethanolamine	Sigma	Sigma, E0135
Sodium Selenite	Sigma	Sigma, S5261
Human Apotranferin	Sigma	Sigma, T2036
Bovine Serum Albumin	Sigma	Sigma, A9647
TrypLE Express	Invitrogen	Invitrogen, 12605
PBS w/o Calcium And Magnesium	Invitrogen	Invitrogen, 14190
Recovery Cell Culture Medium	Invitrogen	Invitrogen, 12648
Centrifuge	Sorvall Legend Xtr	Thermo Fisher Science 75004520
Bioraptr Microfluidic Workstation	Beckmen	-
Pintool	Kalypsys	-
White, TC, Sterile 1536-Well Assay Plates	Greiner Bio-One	Greiner, 789173-F
Viewlux Plate Reader	Perkinelmer	-
T3 (Agonist control compound)	Calbiochem	Calbiochem, 642511
DMSO	Amresco	Kd Medical, Rge-3070
Cell Titer Glo	Promega	Promega, G7572
Tetraoctylammonium bromide	Sigma	Sigma, 294136

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
Recovery Cell Culture Medium	-	-	-	100%
DMEM: F12	90%	100%	90%	-
Fetal Bovine Serum	10%	-	10%	-
Pen/Strep	100U/mL-100µg/mL	-	100U/mL-100µg/mL	-
Insulin	-	10µg/mL	-	-
Ethanolamine	-	10µM	-	-
Sodium Selenite		10ng/mL	-	-
Human Apo-transferrin	-	10µg/mL	-	-
Bovine Serum Albumin	-	500µg/mL	-	-

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a T75 flask

1.2.2 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.
1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.
1.2.4 Transfer the vial contents drop-wise into 10 mL of Thaw Medium in a sterile 15-mL conical tube
1.2.5 Centrifuge cells at 1000 rpm for 4 minutes
1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37°C/5% CO2 incubator
1.2.7 Switch to growth medium at first passage.
1.3. Propagation method
1.3.1 Aspirate medium, rinse once in DPBS, add TrypLE Express (3 mL for a T75 flask and 5 mL for a T175 flask and 7.5 mL for T225 flask) and swirl to coat the cell evenly.
1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.
1.3.3 Centrifuge cells at 1000 rpm for 4 minutes and resuspend in Growth Medium.
1.3.4 Cell should be passage or fed at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture in Growth Medium and resuspend in assay medium
2.2 Dispense 1500 cells/5 µL/well into 1536-well tissue treated white solid plates using a BioRAPTR dispenser.
2.3 After the cells were incubated at 37°C for 4 hours, 23 nL of compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool
2.4 Dispense 1µL of T3 or buffer control using BioRaptr
2.5 Incubate the plates for 23.5 hours at 37°C.
2.6 Add 5µL of Cell Titer Glo to each well using a BioRAPTR dispenser and incubate the plate at room temperature for 30min.
2.7 Measure luminescence using Viewlux

GH3.TRE-Luc cells were dispensed at 1500 cells/4 uL/well into 1536-well white solid bottom plates using a Multidrop Combi dispenser (Thermo Fisher Scientific Inc., Waltham, MA). After the assay plates were incubated at 37 °C for 4 hours, 23 nL of library compound or DMSO controls was transferred to the assay plates by a pintool station (Kalypsys, San Diego, CA), followed by addition of 1 ul of T3 (1 nM, final concentration in the wells) to stimulate TR transactivation. The assay plates were incubated at 37 °C for 23.5 h, and then 1 uL of CellTiter-Fluor reagent (Promega, Madision, WI) of measuring cytotoxicity was added to each well using a Bioraptr Flying Reagent Dispenser (FRD) workstation (Beckman Coulter, Indianapolis, IN, USA). The plates were incubated at 37 °C and 5% CO2 for additional 30 min, and fluorescence intensity was measured by a ViewLux plate reader (PerkinElmer, Shelton, CT).

Proprietary Elements:

This assay is not proprietary; The Tox21 qHTS robotic platform has a 1536-well per run capacity and is capable of fully-automated (hands-free) assay execution (liquid dispensing and aspiration, plate centrifugation and incubation, et cetera) and signal recording (plate readout) [4]. GeneBLAzer® System is publicly available through Invitrogen.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote thyroid receptor mediated gene expression, and is intended to provide

information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

1.3. Assay References

Assay Source Contact Information:

U.S. Tox21 Program
National Center for Advancing Translational Sciences [NCATS]
NIH Chemical Genomics Center [NCGC]
U.S. Environmental Protection Agency [EPA]
National Institutes of Environmental Health Sciences [NIEHS]
National Toxicology Program [NTP]
U.S. Food and Drug Administration [FDA]

Assay Contact: Ruili Huang
NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20850, USA

Assay Publication Year:

2014

Assay Publication:

Freitas, J., Cano, P., Craig-Veit, C., Goodson, M. L., Furlow, J. D., & Murk, A. J. (2011). "Detection of thyroid hormone receptor disruptors by a novel stable in vitro reporter gene assay". Toxicol In Vitro 25(1), 257-266.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

The Tox21 thyroid receptor luciferase GH3 antagonist assay screened a large library of diverse environmental compounds to probe for xenobiotic activity and potential to suppress thyroid-dependent transcription, monitored through decreased luciferase reporter gene signal activation using a TR-luciferase reporter gene construct stimulated by 20µM of the thyroid hormone T3. The assay is run in triplicate on a 1536-well microplate and bioluminescence was measured following 24-hour incubation of cells with test compounds and 30 min incubation of test system with ONE-Glo™ luciferase assay reagent to detect TR inhibition. The bioluminescent signal was monitored using a Promega ViewLux plate reader. Test compounds were selected based on various criteria, e.g., exposure hazard, physicochemical properties, availability and affordability and each assay incorporated 88 chemical duplications (each derived from the same primary stock solution as a sample chemical) and 39 different reference chemicals with known agonistic/antagonistic activities. 10% of the chemicals tested were duplicated chemical structures sourced from separate venders or from different production lots to assess sample variability. Following the incubation period, the cell culture was screened for bioluminescent signals in antagonist mode using luciferase-coupled ATP detection technology. Each compound was tested in a concentration-response format, using 15 concentrations ranging from 1.1 nM to 92 µM. Loss-of-signal due to antagonism was distinguished from cytotoxicity by Compound auto-fluorescence was monitored using auto-fluorescence assays run at interfering wavelengths to allow for filtering of artifacts before analytical endpoint evaluation.

Scientific Principles:

TH is essential for normal brain development both before and after birth and has profound effects on cellular metabolism in almost all organs. One potential mechanism by which endocrine disrupting chemicals may produce toxic effects is by interfering with the ability of thyroid hormones (T3, triiodothyronine and T4, thyroxine). Thyroid hormone interfering compounds can result in neurological disorders by interfering with normal developmental and metabolic processes. Thyroid hormones also have important roles in the initiation and proliferation of central nervous system and cardiovascular tissues. An important component of an endocrine disruptor screening program should be the inclusion of assays designed to screen TH disrupting compounds. The GH3_TRE_LUC cell line is a TR responsive system which is a sensitive and reliable method to evaluate the potential for xenobiotic compounds to act as interfering ligands to the thyroid hormone receptor. This assay is designed to screen a large, structurally diverse chemical library to identify compounds capable of interference with endogenous thyroid signaling by monitoring the increase in luminescent signals relative to a thyroid hormone (T3), positive control and indicator of receptor activation.

Method Development Reference:

Gutleb, A. C., Meerts, I. A., Bergsma, J. H., Schriks, M., & Murk, A. J. (2005). "T-Screen as a tool to identify thyroid hormone receptor active compounds". Environ Toxicol Pharmacol 19(2), 231-238. (PMID: 21783481)

Assay Quality Statistics:

3. Assay Endpoint Descriptions

3.1. Data Interpretation

Biological Response:

Thyroid receptor ligand-binding and antagonism, measured by monitoring decreased luminescence resulting from repressed thyroid response element-driven expression of luciferase

Analytical Elements:

The Tox21_TR_LUC_GH3_Antagonist assay was monitored for decreased luminescence (loss-of-signal) relative to 0.001 mM T3 (positive control, 100% activity) signal, using DMSO (negative control) as a signal baseline, and response was reported as a percent of positive control (T3) activity. Concentration-response relationships were determined based on a range of 15 chemical concentrations. All statistical analyses were conducted using R programming language, employing *tcpl* package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Thyroid receptor activation was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC_{50} (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

ATG_THRa1_TRANS_up
ATG_THRb_TRANS2_up
LTEA_HepaRG_THRSP_up
LTEA_HepaRG_THRSP_dn
NVS_NR_hTRa_Antagonist
Tox21_TR_LUC_GH3_Agonist

3.2. Assay Performance

Assay Performance Measures:

Reference Chemicals / Predictive Capacity:

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking. The chemical inventory used in this assay includes the "e1k" chemical inventory which includes compounds recognized as known estrogen receptor (ER) and androgen receptor (AR) active reference chemicals [9].

4. Assay Documentation

4.1. References

[1] Crofton, K. M. (2008). Int J Androl 31(2): 209-223.
[2] Gutleb, A. C., et al. (2005). Environ Toxicol Pharmacol 19(2): 231-238. (PMID: 21783481)
[3] Hohenwarter, O., et al. (1996). Anal Biochem 234(1): 56-59.
[4] Ghisari, M. and E. C. Bonefeld-Jorgensen (2005). Mol Cell Endocrinol 244(1): 31-41. (PMID: 16221524)
[5] Samuels, H., et al. (1988). J Clin Invest 81(4): 957.
[6] Freitas, J., et al. (2011). Toxicol In Vitro 25(1): 257-266.
[7] Freitas, J., et al. (2014). Current Chem Genom Transl Med 8: 36.
[8] Gauger, K. J., et al. (2007). Environ Health Perspect: 1623-1630.
[9] Richard, A. M., et al. (2016). Chem Res Toxicol Article ASAP. (PMID: 27367298)

4.2. Abbreviations and Definitions

AIC, Akaike Information Criterion
EDC, Endocrine Disrupting Compounds
FBS, Fetal Bovine Serum
FRD, Flying Reagent Dispenser
ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

NICEATM, National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods
NR, Nuclear Receptors
qHTS, Quantitative High-Throughput Screening
T3, Triiodothyronine
T4, Thyroxine
TF, Transcription Factor
TH, Thyroid Hormone
TR, Thyroid Receptor
TRE, Thyroid Hormone Response Elements

4.3. Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)
109 T.W. Alexander Drive (MD-B-205-01)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

3 August 2016

Date of Revisions:

Author of Revisions:

5. Supporting Information

ASSAY DOCUMENTATION

Partial assay information available

ACEA_AR_agonist_80hr

Assay Title: ACEA Biosciences human 22Rv1 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent 22Rv1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	4.02038
Neutral control median absolute deviation, by plate:	0.203708
Positive control well median response value, by plate:	6.91511
Positive control well median absolute deviation, by plate:	0.852776
Negative control well median, by plate:	204.219627901613
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	0.8
SSMD (median across all plates, using positive control wells):	3
SSMD (median across all plates, using negative control wells):	-15
Signal-to-noise (median across all plates, using positive control wells):	13.91
Signal-to-noise (median across all plates, using negative control wells):	-14.77
Signal-to-background (median across all plates, using positive control wells):	1.75
Signal-to-background (median across all plates, using negative control wells):	0.25
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.54436328808604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.6330898642581

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_AR_agonist_AUC_viability

Assay Title: ACEA Biosciences human 22Rv1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent 22Rv1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	173.792
Neutral control median absolute deviation, by plate:	6.74161
Positive control well median response value, by plate:	223.133
Positive control well median absolute deviation, by plate:	20.6158
Negative control well median, by plate:	211.377496416166
Negative control well median absolute deviation value, by plate:	2.6855
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	0.71
SSMD (median across all plates, using positive control wells):	2
SSMD (median across all plates, using negative control wells):	-13
Signal-to-noise (median across all plates, using positive control wells):	8.56
Signal-to-noise (median across all plates, using negative control wells):	-14.36
Signal-to-background (median across all plates, using positive control wells):	1.35
Signal-to-background (median across all plates, using negative control wells):	0.41
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.95647120560566
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.869413616817

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_AR_antagonist_80hr

Assay Title: ACEA Biosciences human 22Rv1 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent 22Rv1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.92602
Neutral control median absolute deviation, by plate:	0.983932
Positive control well median response value, by plate:	4.20221
Positive control well median absolute deviation, by plate:	0.19832
Negative control well median, by plate:	2.87288669770092
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	0.49
SSMD (median across all plates, using positive control wells):	-3
SSMD (median across all plates, using negative control wells):	-6
Signal-to-noise (median across all plates, using positive control wells):	-2.57
Signal-to-noise (median across all plates, using negative control wells):	-5.94
Signal-to-background (median across all plates, using positive control wells):	0.59
Signal-to-background (median across all plates, using negative control wells):	0.14
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.122016065451132
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.366048196353396

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_AR_antagonist_AUC_viability

Assay Title: ACEA Biosciences human 22Rv1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent 22Rv1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	216.845
Neutral control median absolute deviation, by plate:	21.3732
Positive control well median response value, by plate:	170.663
Positive control well median absolute deviation, by plate:	6.34469
Negative control well median, by plate:	175.031543674768
Negative control well median absolute deviation value, by plate:	2.55621
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	0.52
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	-7
Signal-to-noise (median across all plates, using positive control wells):	-2.39
Signal-to-noise (median across all plates, using negative control wells):	-7.16
Signal-to-background (median across all plates, using positive control wells):	0.76
Signal-to-background (median across all plates, using negative control wells):	0.32
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	9.65026114229828
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.9507834268948

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_ER_80hr

Assay Title: ACEA Biosciences human T47D estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ACEA_T47D_80hr was analyzed into 2 assay endpoints. This assay endpoint, ACEA_T47D_80hr_Positive, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of growth reporter, measures of the cells for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene ESR1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T47D cell line

Basic Procedure:
ACEA_ER_80hr, is one of 1 assay component(s) measured or calculated from the ACEA_ER assay. It is designed to make measurements of real-time cell-growth kinetics, a form of growth reporter, as detected with electrical impedance signals by Real-Time Cell Electrode Sensor (RT-CES) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ACEA_ER is a cell-based, single-readout assay that uses T47D, a human breast cell line, with measurements taken at 80 hours after chemical dosing in a 96-well or 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.66702
Neutral control median absolute deviation, by plate:	0.114765
Positive control well median response value, by plate:	3.01195
Positive control well median absolute deviation, by plate:	0.210708
Negative control well median, by plate:	369.450553914196
Negative control well median absolute deviation value, by plate:	0
Z’ (median across all plates, using positive control wells):	0.23
Z’ (median across all plates, using negative control wells):	0.45
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	-5
Signal-to-noise (median across all plates, using positive control wells):	11.34
Signal-to-noise (median across all plates, using negative control wells):	-5.45
Signal-to-background (median across all plates, using positive control wells):	1.79
Signal-to-background (median across all plates, using negative control wells):	0.6
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.95680129019658
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.8704038705897

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

Xing JZ, Zhu L, Gabos S, Xie L. Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. Toxicol In Vitro. 2006 Sep;20(6):995-1004. Epub 2006 Feb 14. PubMed PMID: 16481145.|Rotroff DM, Dix DJ, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Reif DM, Richard AM, Sipes NS, Abassi YA, Jin C, Stampfl M, Judson RS. Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T-47D human ductal carcinoma cells. Chem Res Toxicol. 2013 Jul 15;26(7):1097-107. doi:10.1021/tx400117y. Epub 2013 Jun 10. PubMed PMID: 23682706.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_ER_AUC_viability

Assay Title: ACEA Biosciences human T47D unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ACEA_T47D_80hr was analyzed into 2 assay endpoints. This assay endpoint, ACEA_T47D_AUC_Negative, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of growth reporter, loss-of-signal activity can be used to understand changes in the viability. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T47D cell line

Basic Procedure:
ACEA_ER_AUC_viability, is one of 1 assay component(s) measured or calculated from the ACEA_ER assay. It is designed to make measurements of real-time cell-growth kinetics, a form of growth reporter, as detected with electrical impedance signals by Real-Time Cell Electrode Sensor (RT-CES) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ACEA_ER is a cell-based, single-readout assay that uses T47D, a human breast cell line, with measurements taken at 80 hours after chemical dosing in a 96-well or 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	109.905
Neutral control median absolute deviation, by plate:	4.6305
Positive control well median response value, by plate:	148.898
Positive control well median absolute deviation, by plate:	7.39295
Negative control well median, by plate:	178.368077195595
Negative control well median absolute deviation value, by plate:	3.08298
Z' (median across all plates, using positive control wells):	0.01
Z' (median across all plates, using negative control wells):	0.72
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	-14
Signal-to-noise (median across all plates, using positive control wells):	7.99
Signal-to-noise (median across all plates, using negative control wells):	-18.35
Signal-to-background (median across all plates, using positive control wells):	1.35
Signal-to-background (median across all plates, using negative control wells):	0.17
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.85028963296119
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

Xing JZ, Zhu L, Gabos S, Xie L. Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. Toxicol In Vitro. 2006 Sep;20(6):995-1004. Epub 2006 Feb 14. PubMed PMID: 16481145.|Rotroff DM, Dix DJ, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Reif DM, Richard AM, Sipes NS, Abassi YA, Jin C, Stampfl M, Judson RS. Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T-47D human ductal carcinoma cells. Chem Res Toxicol. 2013 Jul 15;26(7):1097-107. doi:10.1021/tx400117y. Epub 2013 Jun 10. PubMed PMID: 23682706.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_HuRELhumanA_AUC_ABT_48h

Assay Title: ACEA_HuRELhumanA_AUC_ABT_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_HuRELhumanA_AUC_ABT_309h

Assay Title: ACEA_HuRELhumanA_AUC_ABT_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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unspecified

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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ACEA_HuRELhumanA_AUC_Con_309h

Assay Title: ACEA_HuRELhumanA_AUC_Con_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Assay Title: ACEA_HuRELhumanB_AUC_ABT_48h

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1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
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3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_HuRELhumanB_AUC_Con_48h

Assay Title: ACEA_HuRELhumanB_AUC_Con_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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Date of Assay Document Creation:

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ACEA_HuRELhumanB_AUC_Con_309h

Assay Title: ACEA_HuRELhumanB_AUC_Con_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

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unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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ACEA_HuRELhumanC_AUC_ABT_48h

Assay Title: ACEA_HuRELhumanC_AUC_ABT_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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3.2 Assay Performance

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ACEA_HuRELhumanC_AUC_ABT_309h

Assay Title: ACEA_HuRELhumanC_AUC_ABT_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

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Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_HuRELhumanC_AUC_Con_48h

Assay Title: ACEA_HuRELhumanC_AUC_Con_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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Assay Title: ACEA_HuRELhumanC_AUC_Con_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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ACEA_HuRELrat_AUC_ABT_48h

Assay Title: ACEA_HuRELrat_AUC_ABT_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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ACEA_HuRELrat_AUC_ABT_309h

Assay Title: ACEA_HuRELrat_AUC_ABT_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Signal-to-background (median across all plates, using negative control wells):	unspecified
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3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_HuRELrat_AUC_Con_48h

Assay Title: ACEA_HuRELrat_AUC_Con_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_HuRELrat_AUC_Con_309h

Assay Title: ACEA_HuRELrat_AUC_Con_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_SERM_Agonist_ECC-1_72hr

Assay Title: ACEA Biosciences human ECC-1 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent ECC-1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_SERM_Agonist_Ishikawa_72hr

Assay Title: ACEA Biosciences human Ishikawa estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent Ishikawa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_SERM_Agonist_LNCaP_72hr

Assay Title: ACEA Biosciences human LNCaP estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent LNCaP cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_SERM_Antagonist_ECC-1_72hr

Assay Title: ACEA Biosciences human ECC-1 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent ECC-1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_SERM_Antagonist_Ishikawa_72hr

Assay Title: ACEA Biosciences human Ishikawa estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent Ishikawa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ACEA_SERM_Antagonist_LNCaP_72hr

Assay Title: ACEA Biosciences human LNCaP estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent LNCaP cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_1_24hr_dn

Assay Title: APR_Cell_count_1_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.065
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.059304
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.59304

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_1_24hr_up

Assay Title: APR_Cell_count_1_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.8735
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.059304
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.59304

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_1_72hr_dn

Assay Title: APR_Cell_count_1_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.135
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0822843
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.822843

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_1_72hr_up

Assay Title: APR_Cell_count_1_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.99451
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0822843
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.822843

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_2_24hr_dn

Assay Title: APR_Cell_count_2_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.0800000000000001
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0681996000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.681996000000001

Chemical Library Scope and Limitations:

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unspecified

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DMSO, Dimethyl Sulfoxide

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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APR_Cell_count_2_24hr_up

Assay Title: APR_Cell_count_2_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.945
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0681996000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.681996000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_2_72hr_dn

Assay Title: APR_Cell_count_2_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.71
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1616034
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.616034

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_count_2_72hr_up

Assay Title: APR_Cell_count_2_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.998835
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1616034
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.616034

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_1_24hr_dn

Assay Title: APR_Cell_cycle_arrest_1_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.86
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0652343999999999
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.652343999999999

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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APR_Cell_cycle_arrest_1_24hr_up

Assay Title: APR_Cell_cycle_arrest_1_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.711
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0652343999999999
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.652343999999999

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_1_72hr_dn

Assay Title: APR_Cell_cycle_arrest_1_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	9.185
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0889560000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.889560000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_1_72hr_up

Assay Title: APR_Cell_cycle_arrest_1_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0889560000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.889560000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_2_24hr_dn

Assay Title: APR_Cell_cycle_arrest_2_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.54
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0948864
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.948864

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_2_24hr_up

Assay Title: APR_Cell_cycle_arrest_2_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.7915
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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3.2 Assay Performance

Assay Performance Measures:

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Standard maximum concentration tested:	199.999999999999 µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.948864

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4.1 Resources

Assay Source Information:

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unspecified

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_2_72hr_dn

Assay Title: APR_Cell_cycle_arrest_2_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.14
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.066717
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.66717

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Cell_cycle_arrest_2_72hr_up

Assay Title: APR_Cell_cycle_arrest_2_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Apoptosis_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.97212810498155
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.130219759769282
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.30219759769282

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Apoptosis_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.83805848253752
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Apoptosis_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.23257277656758
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.127878185982627
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.27878185982627

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Apoptosis_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.54629723619409
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.127878185982627
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.27878185982627

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Apoptosis_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.33589631806317
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.132838805603301
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.32838805603301

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Apoptosis_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.16097650632998
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_CellLoss_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.35864853533757
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.103255550775048
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03255550775048

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_CellLoss_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.461803851417101
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.103255550775048
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03255550775048

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AIC, Akaike Information Criterion

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_CellLoss_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.19094496074758
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0849998387382719
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.849998387382719

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_CellLoss_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.381037858863129
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0849998387382719
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.849998387382719

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_CellLoss_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.57751605368857
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0998290273104093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.998290273104093

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_CellLoss_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.512682437028805
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0998290273104093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.998290273104093

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNADamage_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.34901116490139
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.111041214475855
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.11041214475855

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNADamage_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.24023138316127
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.111041214475855
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.11041214475855

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNADamage_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.32255015084537
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.131274031028898
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.31274031028898

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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EDC, Endocrine disrupting chemicals

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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APR_Hepat_DNADamage_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.02076915231817
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.131274031028898
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.31274031028898

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNADamage_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.20718078625406
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.161176127862192
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.61176127862192

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNADamage_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.5769747920704
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.161176127862192
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.61176127862192

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNATexture_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.66897980798963
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0786878782392633
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.786878782392633

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNATexture_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.54680873000686
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0786878782392633
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.786878782392633

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNATexture_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.83975086411954
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0765385007962508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.765385007962508

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNATexture_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.40444553575868
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0765385007962508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.765385007962508

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNATexture_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.598945715546893
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.101137632873487
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01137632873487

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_DNATexture_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.02713033728032
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01137632873487

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_LysosomalMass_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.07516924740593
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.105292431380499
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.05292431380499

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_LysosomalMass_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.686640015421828
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.105292431380499
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_LysosomalMass_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.986363627064687
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0742415979473183
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.742415979473183

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_LysosomalMass_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.26593276358748
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

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4.1 Resources

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_LysosomalMass_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.24463820105879
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0986926284909225
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.986926284909225

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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SSMD, Strictly Standardized Mean Difference

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_LysosomalMass_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.10762576546193
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0986926284909225
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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnl_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.36821004874421
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0384676600046139
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.384676600046139

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_1hr_MEAN_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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Assay Title: Apredica rat hepatocyte unspecified assay

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1.1 Overview

Assay Summary:

unspecified

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Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
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Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnl_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.370938355762183
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0384676600046139
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.384676600046139

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.23627409054888
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00896050582667891
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_24hr_MEAN_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_24hr_MEAN_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnl_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.13224883438768
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00896050582667891
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.73476302224582
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0148810027674112
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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CYP, Cytochrome P450s

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_48hr_MEAN_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnI_48hr_MEAN_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_MitoFxnl_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.542799603990821
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0148810027674112
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_NuclearSize_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.204719704617376
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0119028517187559
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_NuclearSize_1hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.156922357641431
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0119028517187559
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_NuclearSize_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.518390790945251
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0182873897891468
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_NuclearSize_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.126813853542735
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0182873897891468
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_NuclearSize_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.570572055236332
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0226416107598692
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_NuclearSize_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.167587485043768
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0226416107598692
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_1hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.7794800794383
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.165732738930588
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.65732738930588

Chemical Library Scope and Limitations:

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1.1 Overview

Assay Summary:

unspecified

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Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
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Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.61120950600362
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.165732738930588
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.65732738930588

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_24hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.94315317426651
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.158409538015371
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.58409538015371

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_24hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.80861319321396
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.158409538015371
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.58409538015371

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_48hr_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.66537514054208
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.17904929283415
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.7904929283415

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_48hr_TotalArea_dn

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_48hr_TotalArea_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Hepat_Steatosis_48hr_up

Assay Title: Apredica rat hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.90179496835703
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.17904929283415
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.7904929283415

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellCycleArrest_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellCycleArrest_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellCycleArrest_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellCycleArrest_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.36048492366783
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0724916078061459
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.724916078061459

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellCycleArrest_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellCycleArrest_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellCycleArrest_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "arrest".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellCycleArrest_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.83170978527701
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0724916078061459
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.724916078061459

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellCycleArrest_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellCycleArrest_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellCycleArrest_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellCycleArrest_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.36646305054309
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.070192536163885
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.70192536163885

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellCycleArrest_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellCycleArrest_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellCycleArrest_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "arrest".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellCycleArrest_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.88126211987634
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.070192536163885
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.70192536163885

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellCycleArrest_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellCycleArrest_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellCycleArrest_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellCycleArrest_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	9.05110426358
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0927628116998214
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.927628116998214

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellCycleArrest_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellCycleArrest_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellCycleArrest_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "arrest".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellCycleArrest_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.41900660186014
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0927628116998214
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.927628116998214

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellLoss_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellLoss_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellLoss_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellLoss_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.50015883252734
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0556971310444626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.556971310444626

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellLoss_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellLoss_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellLoss_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellLoss_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.155891924494857
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0556971310444626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.556971310444626

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellLoss_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellLoss_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellLoss_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellLoss_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	8.53702901338042
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0663298579828901
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.663298579828901

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellLoss_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellLoss_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellLoss_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellLoss_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.220295546369459
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0663298579828901
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.663298579828901

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellLoss_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellLoss_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellLoss_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellLoss_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	9.11326661901081
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0889173575579604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.889173575579604

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_CellLoss_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_CellLoss_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_CellLoss_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_CellLoss_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.516147918777242
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0889173575579604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.889173575579604

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MicrotubuleCSK_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MicrotubuleCSK_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MicrotubuleCSK_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of conformation reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the cellular-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MicrotubuleCSK_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of protein conformation, a form of conformation reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.04127175305774
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.123592271242576
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.23592271242576

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MicrotubuleCSK_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MicrotubuleCSK_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MicrotubuleCSK_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of conformation reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the cellular-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MicrotubuleCSK_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of protein conformation, a form of conformation reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.27254648616166
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.123592271242576
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.23592271242576

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MicrotubuleCSK_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MicrotubuleCSK_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MicrotubuleCSK_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of conformation reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the cellular-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MicrotubuleCSK_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of protein conformation, a form of conformation reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.59336970556547
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0811664042375869
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.811664042375869

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MicrotubuleCSK_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MicrotubuleCSK_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MicrotubuleCSK_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of conformation reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the cellular-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MicrotubuleCSK_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of protein conformation, a form of conformation reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.28301125078827
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0811664042375869
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.811664042375869

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MicrotubuleCSK_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MicrotubuleCSK_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MicrotubuleCSK_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of conformation reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the cellular-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MicrotubuleCSK_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of protein conformation, a form of conformation reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.79728608742584
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.103090960787123
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03090960787123

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MicrotubuleCSK_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MicrotubuleCSK_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MicrotubuleCSK_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of conformation reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the cellular-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MicrotubuleCSK_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of protein conformation, a form of conformation reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.46907288595208
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.103090960787123
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03090960787123

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMass_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMass_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMass_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMass_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.09488309228565
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0356082721804674
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.356082721804674

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMass_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMass_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMass_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMass_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.256267681639147
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0356082721804674
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.356082721804674

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMass_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMass_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMass_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMass_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.73861523833675
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0496026277776128
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.496026277776128

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMass_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMass_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMass_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMass_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.13139582683717
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0496026277776128
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.496026277776128

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMass_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMass_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMass_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMass_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.81715665845697
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0684622892579271
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.684622892579271

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMass_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMass_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMass_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMass_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.84170592403691
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMembPot_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMembPot_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMembPot_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMembPot_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.89066763977763
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.17865289567093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.7865289567093

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMembPot_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMembPot_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMembPot_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMembPot_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.18037215557646
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.17865289567093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.7865289567093

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMembPot_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMembPot_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMembPot_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMembPot_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.98928270916676
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0838241022789054
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.838241022789054

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMembPot_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMembPot_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMembPot_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMembPot_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMembPot_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMembPot_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMembPot_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMembPot_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.29600345304595
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.073345060485155
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.73345060485155

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoMembPot_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoMembPot_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoMembPot_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoMembPot_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.88063538344502
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.073345060485155
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.73345060485155

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoticArrest_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoticArrest_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoticArrest_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoticArrest_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.697680000234759
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.117847601257847
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17847601257847

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoticArrest_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoticArrest_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoticArrest_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "arrest".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoticArrest_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.25160586654058
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.117847601257847
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17847601257847

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoticArrest_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoticArrest_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoticArrest_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoticArrest_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.88860078290181
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.109371378535482
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09371378535482

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoticArrest_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoticArrest_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoticArrest_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "arrest".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoticArrest_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.53232300687818
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.109371378535482
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09371378535482

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoticArrest_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoticArrest_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoticArrest_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoticArrest_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.40683420210385
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.141906560088972
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.41906560088972

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_MitoticArrest_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_MitoticArrest_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_MitoticArrest_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "arrest".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_MitoticArrest_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.19665639988156
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.141906560088972
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.41906560088972

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_NuclearSize_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_NuclearSize_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_NuclearSize_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the signaling at the nuclear-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_NuclearSize_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.432939021819874
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00761052381278775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_NuclearSize_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_NuclearSize_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_NuclearSize_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the signaling at the nuclear-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_NuclearSize_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.069993885601485
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00761052381278775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_NuclearSize_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_NuclearSize_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_NuclearSize_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the signaling at the nuclear-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_NuclearSize_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.826239421032209
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0101836771658257
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_NuclearSize_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_NuclearSize_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_NuclearSize_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the signaling at the nuclear-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_NuclearSize_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.410125952228698
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0101836771658257
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_NuclearSize_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_NuclearSize_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_NuclearSize_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for loss-of-signal activity can be used to understand the signaling at the nuclear-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_NuclearSize_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.597569724757852
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0147358894772317
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_NuclearSize_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_NuclearSize_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_NuclearSize_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, measures of all nuclear dna for gain-of-signal activity can be used to understand the signaling at the nuclear-level. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "organelle conformation".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_NuclearSize_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.560656171550777
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0147358894772317
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_OxidativeStress_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_OxidativeStress_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_OxidativeStress_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_OxidativeStress_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.766611171875041
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0771552014027464
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.771552014027464

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_OxidativeStress_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_OxidativeStress_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_OxidativeStress_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_OxidativeStress_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.48717777019422
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0771552014027464
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.771552014027464

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

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Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_OxidativeStress_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_OxidativeStress_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_OxidativeStress_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_OxidativeStress_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.639762277388676
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0818584158091634
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.818584158091634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_OxidativeStress_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_OxidativeStress_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_OxidativeStress_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_OxidativeStress_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.73857374257648
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0818584158091634
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.818584158091634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_OxidativeStress_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_OxidativeStress_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_OxidativeStress_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_OxidativeStress_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.833660867477996
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.109533469841783
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09533469841783

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_OxidativeStress_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_OxidativeStress_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_OxidativeStress_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_OxidativeStress_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.39752564981621
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.109533469841783
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09533469841783

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_p53Act_1h_dn

Assay Title: Apredica human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_p53Act_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_p53Act_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_p53Act_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.665876878781834
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0842717676937473
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.842717676937473

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_p53Act_1h_up

Assay Title: Apredica human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_p53Act_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_p53Act_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_p53Act_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.50983141319747
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	1
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0842717676937473
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_p53Act_24h_dn

Assay Title: Apredica human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_p53Act_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_p53Act_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_p53Act_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.98782798570558
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0972268260844447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.972268260844447

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

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Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_p53Act_24h_up

Assay Title: Apredica human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_p53Act_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_p53Act_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_p53Act_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.21347278836369
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0972268260844447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.972268260844447

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_p53Act_72h_dn

Assay Title: Apredica human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_p53Act_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_p53Act_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_p53Act_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.04621676259055
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.118088888381775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.18088888381775

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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APR_HepG2_p53Act_72h_up

Assay Title: Apredica human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_p53Act_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_p53Act_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_p53Act_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of dna content, a form of viability reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.16582917169497
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.118088888381775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.18088888381775

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_StressKinase_1h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_StressKinase_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_StressKinase_1h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_StressKinase_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.810034901983084
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0791420108970909
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.791420108970909

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_StressKinase_1h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_StressKinase_1hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_StressKinase_1h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_StressKinase_1hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_1hr assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_1hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.64397744091081
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0791420108970909
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.791420108970909

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_StressKinase_24h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_StressKinase_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_StressKinase_24h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_StressKinase_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.21509308676649
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0922739427386406
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.922739427386406

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_StressKinase_24h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_StressKinase_24hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_StressKinase_24h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_StressKinase_24hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_24hr assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_24hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.59370815515596
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0922739427386406
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.922739427386406

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_StressKinase_72h_dn

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_StressKinase_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_StressKinase_72h_dn, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, measures of protein for loss-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_StressKinase_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.08098309219362
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.108799248027028
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.08799248027028

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_HepG2_StressKinase_72h_up

Assay Title: Apredica human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component APR_HepG2_StressKinase_72hr was analyzed into 2 assay endpoints. This assay endpoint, APR_HepG2_StressKinase_72h_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, measures of protein for gain-of-signal activity can be used to understand the signaling at the pathway-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "stress response".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
APR_HepG2_StressKinase_72hr, is one of 10 assay component(s) measured or calculated from the APR_HepG2_72hr assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by HCS Fluorescent Imaging technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

APR_HepG2_72hr is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 72 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.92194644315686
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.108799248027028
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.08799248027028

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

Giuliano KA, Gough AH, Taylor DL, Vernetti LA, Johnston PA. Early safety assessment using cellular systems biology yields insights into mechanisms of action. J Biomol Screen. 2010 Aug;15(7):783-97. doi: 10.1177/1087057110376413. Epub 2010 Jul 16. PubMed PMID: 20639501.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Mitochondrial_mass_24hr_dn

Assay Title: APR_Mitochondrial_mass_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.345
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0452193
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.452193

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Mitochondrial_mass_24hr_up

Assay Title: APR_Mitochondrial_mass_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.93415
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0452193
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.452193

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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384 well

Experimental System:

unspecified

Basic Procedure:
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Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.455
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0459606
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.459606

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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CYP, Cytochrome P450s

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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1.1 Overview

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unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.9826
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

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Assay Performance Measures:

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1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.0700000000000001
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.467019

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Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
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unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0467019
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.467019

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Mitochondrial_membrane_potential_72hr_dn

Assay Title: APR_Mitochondrial_membrane_potential_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.035
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.029652
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.29652

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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APR_Mitochondrial_membrane_potential_72hr_up

Assay Title: APR_Mitochondrial_membrane_potential_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.8965
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.029652
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.29652

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Nuclear_size_1_24hr_dn

Assay Title: APR_Nuclear_size_1_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.14
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.014826
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Nuclear_size_1_24hr_up

Assay Title: APR_Nuclear_size_1_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.2895
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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4.1 Resources

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unspecified

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Nuclear_size_1_72hr_dn

Assay Title: APR_Nuclear_size_1_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.195
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0200151
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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APR_Nuclear_size_1_72hr_up

Assay Title: APR_Nuclear_size_1_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.32
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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APR_Nuclear_size_2_24hr_dn

Assay Title: APR_Nuclear_size_2_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.165
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0163086
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Nuclear_size_2_24hr_up

Assay Title: APR_Nuclear_size_2_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.3125
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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MIE, Molecular Initiating Event

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Assay Summary:

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1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

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Caveats:

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Method Updates / Confirmatory Studies:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.185
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0214977
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Nuclear_size_2_72hr_up

Assay Title: APR_Nuclear_size_2_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.378
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0214977
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p53_activation_24hr_dn

Assay Title: APR_p53_activation_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.95
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0778365000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.778365000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p53_activation_24hr_up

Assay Title: APR_p53_activation_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.823
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0778365000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.778365000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p53_activation_72hr_dn

Assay Title: APR_p53_activation_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	14.35
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1578969
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.578969

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p53_activation_72hr_up

Assay Title: APR_p53_activation_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.647
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1578969
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.578969

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Author of Revisions:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-cJun_24hr_dn

Assay Title: APR_p-cJun_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.645
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0733887
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.733887

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-cJun_24hr_up

Assay Title: APR_p-cJun_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.1285
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0733887
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.733887

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-cJun_72hr_dn

Assay Title: APR_p-cJun_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.49
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0785778000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.785778000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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APR_p-cJun_72hr_up

Assay Title: APR_p-cJun_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.296
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0785778000000001
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.785778000000001

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H2AX_24hr_dn

Assay Title: APR_p-H2AX_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	8.705
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0652344
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.652344

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H2AX_24hr_up

Assay Title: APR_p-H2AX_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.117
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0652344
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.652344

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H2AX_72hr_dn

Assay Title: APR_p-H2AX_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	9.85
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0971103
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.971103

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H2AX_72hr_up

Assay Title: APR_p-H2AX_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.133
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Standard maximum concentration tested:	199.999999999999 µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.971103

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H3_24hr_dn

Assay Title: APR_p-H3_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	11.3
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1171254
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.171254

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H3_24hr_up

Assay Title: APR_p-H3_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.94365
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.171254

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H3_72hr_dn

Assay Title: APR_p-H3_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	18.35
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1223145
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.223145

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_p-H3_72hr_up

Assay Title: APR_p-H3_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.99703
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.223145

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Tubulin_microtubule_stability_24hr_dn

Assay Title: APR_Tubulin_microtubule_stability_24hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.935
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0437367
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.437367

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Tubulin_microtubule_stability_24hr_up

Assay Title: APR_Tubulin_microtubule_stability_24hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.5615
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0437367
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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Tubulin_microtubule_stability_72hr_dn

Assay Title: APR_Tubulin_microtubule_stability_72hr_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.8
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	10
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0904386
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.904386

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

APR_Tubulin_microtubule_stability_72hr_up

Assay Title: APR_Tubulin_microtubule_stability_72hr_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.7005
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0904386
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.904386

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Apredica, a part of Cyprotex, is a preclinical Contract Research Organization (CRO) that provides services including the CellCiphr High Content Screening system.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNC_CT

Assay Title: ArunA Biomedical human H9-derived embryonic neural crest (hNC) stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic neural crest (hNC) stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNC_MG_dn

Assay Title: ArunA Biomedical human H9-derived embryonic neural crest (hNC) stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic neural crest (hNC) stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNC_MG_up

Assay Title: ArunA Biomedical human H9-derived embryonic neural crest (hNC) stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic neural crest (hNC) stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_BranchPtsperNeuron_dn

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_BranchPtsperNeuron_up

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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MIE, Molecular Initiating Event

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_NeuriteCountperNeuron_dn

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_NeuriteCountperNeuron_up

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_NeuriteLengthperNeuron_dn

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_NeuriteLengthperNeuron_up

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_NucleusCount_dn

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNN_NO_NucleusCount_up

Assay Title: ArunA Biomedical human H9-derived embryonic differentiated neurons (hN2 v2.0) unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic differentiated neurons (hN2 v2.0) cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNP_CT

Assay Title: ArunA Biomedical human H9-derived neuroprogenitor (hNP) stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived neuroprogenitor (hNP) stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNP_MG_dn

Assay Title: ArunA Biomedical human H9-derived embryonic neuroprogenitor (hNP) stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic neuroprogenitor (hNP) stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ARUNA_H9_hNP_MG_up

Assay Title: ArunA Biomedical human H9-derived embryonic neuroprogenitor (hNP) stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H9-derived embryonic neuroprogenitor (hNP) stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Ahr_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Ahr_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Ahr_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.34
Neutral control median absolute deviation, by plate:	0.151225
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.35640412360734
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.44

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.198735799532917
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.993678997664585

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Ahr_CIS_up

Assay Title: Attagene human HepG2 aryl hydrocarbon receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Ahr_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Ahr_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene AHR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic helix-loop-helix protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Ahr_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.34
Neutral control median absolute deviation, by plate:	0.151225
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.41339159598394
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.44

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.198735799532917
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.993678997664585

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_AP_1_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_AP_1_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_AP_1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.473
Neutral control median absolute deviation, by plate:	0.145295
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.95928743182687
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.118905956632889
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.594529783164445

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_AP_1_CIS_up

Assay Title: Attagene human HepG2 FBJ murine osteosarcoma viral oncogene homolog|jun proto-oncogene assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_AP_1_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_AP_1_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene FOS and JUN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_AP_1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.473
Neutral control median absolute deviation, by plate:	0.145295
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.26854699855934
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.118905956632889
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.594529783164445

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_AP_2_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_AP_2_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_AP_2_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.31
Neutral control median absolute deviation, by plate:	0.160121
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.55660027764429
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0641769994651269
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.320884997325634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_AP_2_CIS_up

Assay Title: Attagene human HepG2 transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)|transcription factor AP-2 beta (activating enhancer binding protein 2 beta)|transcription factor AP-2 delta (activating enhancer binding protein 2 delta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_AP_2_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_AP_2_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene TFAP2A and TFAP2B and TFAP2D. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic helix-turn-helix leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_AP_2_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long

term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.31
Neutral control median absolute deviation, by plate:	0.160121
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.99550211626041
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0641769994651269
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.320884997325634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_AR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_AR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_AR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.1575
Neutral control median absolute deviation, by plate:	0.264644
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.84620266890942
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.176128357268682
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.88064178634341

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_BRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_BRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_BRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.255
Neutral control median absolute deviation, by plate:	0.0993342
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.53278048608639
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.39

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.180639156705349
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.903195783526745

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_BRE_CIS_up

Assay Title: Attagene human HepG2 SMAD family member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_BRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_BRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene SMAD1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "Smad protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_BRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.255
Neutral control median absolute deviation, by plate:	0.0993342
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.88006232570067
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.39

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.180639156705349
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.903195783526745

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_C_EBP_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_C_EBP_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_C_EBP_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.173
Neutral control median absolute deviation, by plate:	0.269833
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.90566680587051
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.102363128245752
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.51181564122876

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_C_EBP_CIS_up

Assay Title: Attagene human HepG2 CCAAT/enhancer binding protein (C/EBP), beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_C_EBP_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_C_EBP_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene CEBPB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_C_EBP_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.173
Neutral control median absolute deviation, by plate:	0.269833
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.87827334024846
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.102363128245752
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.51181564122876

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_CAR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_CAR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_CAR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.369
Neutral control median absolute deviation, by plate:	1.18905
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.8269375461384
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.205743115892689
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.02871557946345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_CAR_TRANS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group I, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_CAR_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_CAR_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1I3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_CAR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.369
Neutral control median absolute deviation, by plate:	1.18905
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.30821174588902
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.205743115892689
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.02871557946345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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ATCC, American Tissue Culture Collection

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chAR_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chAR_TRANS_XSP1_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chAR_XSP2_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chAR_XSP2_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chERa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chERa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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EPA NCCT

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ATG_chERa_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_chERa_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 (chicken)

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_CMV_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_CMV_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_CMV_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.46
Neutral control median absolute deviation, by plate:	0.139364
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.87493744937323
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.134906319041969
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.674531595209845

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_CMV_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_CMV_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_CMV_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_CMV_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.46
Neutral control median absolute deviation, by plate:	0.139364
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.96748718648151
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.134906319041969
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.674531595209845

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_COUP_TF1_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_COUP_TF1_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.385
Neutral control median absolute deviation, by plate:	0.0348411
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.47193022305366
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.324521857984647
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.62260928992323

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_COUP_TF1_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group F, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.385
Neutral control median absolute deviation, by plate:	0.0348411
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.480409161206381
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.324521857984647
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.62260928992323

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_COUP_TF2_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_COUP_TF2_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.581
Neutral control median absolute deviation, by plate:	0.0644931
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.877001520299979
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.236105992419672
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.18052996209836

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_COUP_TF2_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group F, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.581
Neutral control median absolute deviation, by plate:	0.0644931
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.426133154516436
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.18052996209836

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_CRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_CRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_CRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.545
Neutral control median absolute deviation, by plate:	0.180877
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.46694958792131
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.33

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.145628192303698
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.72814096151849

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_CRE_CIS_up

Assay Title: Attagene human HepG2 cAMP responsive element binding protein 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_CRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_CRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene CREB3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_CRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.545
Neutral control median absolute deviation, by plate:	0.180877
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.80479240798978
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.33

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.145628192303698
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.72814096151849

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_DAX1_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_DAX1_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.777
Neutral control median absolute deviation, by plate:	0.0756126
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.514785191827839
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.194186603933561
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.970933019667805

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_DAX1_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 0, group B, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.777
Neutral control median absolute deviation, by plate:	0.0756126
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.430636404948015
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.194186603933561
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.970933019667805

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_DR4_LXR_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_DR4_LXR_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_DR4_LXR_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.044
Neutral control median absolute deviation, by plate:	0.333585
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.00630897211145
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.117651066211554
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.58825533105777

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_DR4_LXR_CIS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group H, member 2|nuclear receptor subfamily 1, group H, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_DR4_LXR_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_DR4_LXR_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1H2 and NR1H3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_DR4_LXR_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.044
Neutral control median absolute deviation, by plate:	0.333585
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.91784014778279
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.117651066211554
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.58825533105777

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_DR5_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_DR5_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_DR5_RAR_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.482
Neutral control median absolute deviation, by plate:	0.0845082
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.52004285579791
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.118457077090722
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.59228538545361

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_DR5_CIS_up

Assay Title: Attagene human HepG2 retinoic acid receptor, alpha|retinoic acid receptor, beta|retinoic acid receptor, gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_DR5_RAR_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_DR5_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RARA and RARB and RARG. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_DR5_RAR_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.482
Neutral control median absolute deviation, by plate:	0.0845082
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.33567232915563
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.118457077090722
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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_E_Box_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_E_Box_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_E_Box_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.939
Neutral control median absolute deviation, by plate:	0.241664
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.86223882913073
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0945444848252286
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.472722424126143

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_E_Box_CIS_up

Assay Title: Attagene human HepG2 upstream transcription factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_E_Box_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_E_Box_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene USF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic helix-loop-helix protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_E_Box_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.939
Neutral control median absolute deviation, by plate:	0.241664
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.8306800289884
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0945444848252286
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.472722424126143

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_E2F_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_E2F_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_E2F_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.296
Neutral control median absolute deviation, by plate:	0.029652
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.16929869579285
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0965371896306431
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.482685948153216

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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25 September 2018

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_E2F_CIS_up

Assay Title: Attagene human HepG2 E2F transcription factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_E2F_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_E2F_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene E2F1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "E2F transcription factor".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_E2F_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.296
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Negative control well median, by plate:	1.58699018206814
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0965371896306431
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.482685948153216

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_EAR2_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_EAR2_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.294
Neutral control median absolute deviation, by plate:	0.0385476
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.83536126222207
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.272088525834437
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.36044262917218

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_EAR2_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group F, member 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.294
Neutral control median absolute deviation, by plate:	0.0385476
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.442115639691417
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.272088525834437
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.36044262917218

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_EGR_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_EGR_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_EGR_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.366
Neutral control median absolute deviation, by plate:	0.0756126
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.0566377151132
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.21

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.13830352367301
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.69151761836505

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_EGR_CIS_up

Assay Title: Attagene human HepG2 early growth response 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_EGR_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_EGR_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene EGR1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "zinc finger".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_EGR_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.366
Neutral control median absolute deviation, by plate:	0.0756126
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.60445413482149
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.21

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.13830352367301
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.69151761836505

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ERa_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ERa_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_ERa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.7745
Neutral control median absolute deviation, by plate:	0.496671
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.88050673000402
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.28

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.229925666350804
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.14962833175402

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ERb_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ERb_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.6625
Neutral control median absolute deviation, by plate:	0.0911799
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.9644103392643
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.212520090060912
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.06260045030456

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ERE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ERE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_ERE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.882
Neutral control median absolute deviation, by plate:	0.185325
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.8055771090897
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.21

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.104866586738015
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.524332933690075

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ERRa_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ERRa_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_ERRa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.4545
Neutral control median absolute deviation, by plate:	0.399561
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.18636535461186
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.239597209915871
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.19798604957936

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ERRb_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ERRb_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.7155
Neutral control median absolute deviation, by plate:	0.478138
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.604818812471047
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.28

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.456823393751574
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	2.28411696875787

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ERRg_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ERRg_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_ERRg_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.3135
Neutral control median absolute deviation, by plate:	1.56118
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.70373229098345
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.47

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.277261633333122
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.38630816666561

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Ets_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Ets_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Ets_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.723
Neutral control median absolute deviation, by plate:	0.0681996
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.7588498640085
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0728368015003483
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.364184007501742

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Ets_CIS_up

Assay Title: Attagene human HepG2 v-ets avian erythroblastosis virus E26 oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Ets_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Ets_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene ETS1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "winged helix-turn-helix".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Ets_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.723
Neutral control median absolute deviation, by plate:	0.0681996
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.35682645556111
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0728368015003483
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.364184007501742

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_FoxA2_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_FoxA2_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_FoxA2_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.827
Neutral control median absolute deviation, by plate:	0.0934038
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.21361628385221
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0973822773985102
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.486911386992551

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_FoxA2_CIS_up

Assay Title: Attagene human HepG2 forkhead box A2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_FoxA2_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_FoxA2_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene FOXA2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "forkhead box protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_FoxA2_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.827
Neutral control median absolute deviation, by plate:	0.0934038
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.73487489975508
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0973822773985102
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.486911386992551

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_FoxO_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_FoxO_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_FoxO_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.387
Neutral control median absolute deviation, by plate:	0.029652
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.12384913089434
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0948027508729423
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.474013754364712

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_FoxO_CIS_up

Assay Title: Attagene human HepG2 forkhead box O1|forkhead box O3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_FoxO_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_FoxO_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene FOXO1 and FOXO3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "forkhead box protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_FoxO_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.387
Neutral control median absolute deviation, by plate:	0.029652
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.32827892703975
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0948027508729423
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.474013754364712

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frAR_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 androgen receptor L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frAR_TRANS_XSP1_up

Assay Title: Attagene human HepG2 androgen receptor L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frAR_XSP2_dn

Assay Title: Attagene human HepG2 androgen receptor L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

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ATG_frAR_XSP2_up

Assay Title: Attagene human HepG2 androgen receptor L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER1_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER1_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 1 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER1_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER1_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 1 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 L homeolog [Xenopus laevis (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER2_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 2 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2 L homeolog [Â Xenopus laevisÂ (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER2_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 2 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2 L homeolog [Â Xenopus laevisÂ (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER2_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 2 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2 L homeolog [Â Xenopus laevisÂ (African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frER2_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 2 L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2 L homeolog [Â Xenopus laevisÂ (African clawed frog)]

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

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References:

unspecified

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frTRa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha L homeolog [Â Xenopus laevis(African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frTRa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha L homeolog [Â Xenopus laevis(African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frTRa_XSP2_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha L homeolog [Â Xenopus laevis(African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_frTRa_XSP2_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha L homeolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha L homeolog [Â Xenopus laevis(African clawed frog)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_FXR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_FXR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_FXR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.2395
Neutral control median absolute deviation, by plate:	0.286142
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.2199929787218
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.169193582430729
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.845967912153645

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GAL4_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GAL4_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GAL4_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.792
Neutral control median absolute deviation, by plate:	0.473691
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.50728313689928
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.157309704731119
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.786548523655595

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GAL4_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_GAL4_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_GAL4_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "baseline control".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GAL4_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.792
Neutral control median absolute deviation, by plate:	0.473691
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.7794501918267
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.157309704731119
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.786548523655595

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GAL4_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GAL4_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GAL4_XSP2_dn

Assay Title: Attagene human HepG2 Gal4p assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GAL4_XSP2_up

Assay Title: Attagene human HepG2 Gal4p assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GATA_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GATA_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GATA_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.746
Neutral control median absolute deviation, by plate:	0.0459606
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.11022531849706
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0658127645146236
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.329063822573118

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GATA_CIS_up

Assay Title: Attagene human HepG2 GATA binding protein 1 (globin transcription factor 1) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_GATA_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_GATA_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene GATA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "GATA proteins".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GATA_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.746
Neutral control median absolute deviation, by plate:	0.0459606
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.65430285420771
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0658127645146236
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.329063822573118

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GCNF_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GCNF_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.4395
Neutral control median absolute deviation, by plate:	0.0726474
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.22302684486191
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.17

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.27843118375746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.3921559187873

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GCNF_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 6, group A, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.4395
Neutral control median absolute deviation, by plate:	0.0726474
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.54613350191262
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.17

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.27843118375746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.3921559187873

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GLI_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GLI_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GLI_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.532
Neutral control median absolute deviation, by plate:	0.0429954
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.05889368905357
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0775451524156662
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.387725762078331

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GLI_CIS_up

Assay Title: Attagene human HepG2 GLI family zinc finger 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_GLI_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_GLI_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene GLI1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "zinc finger".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GLI_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.532
Neutral control median absolute deviation, by plate:	0.0429954
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.23311186392244
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0775451524156662
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.387725762078331

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADORA2A_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_ADORA2A_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	11.9385
Neutral control median absolute deviation, by plate:	2.42776
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.438563622853276
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.243514895470674
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.21757447735337

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADORA2A_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	11.9385
Neutral control median absolute deviation, by plate:	2.42776
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.69347401868245
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.243514895470674
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.21757447735337

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADORA2B_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_ADORA2B_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	5.595
Neutral control median absolute deviation, by plate:	0.851012
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.388179742028337
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.273962350744444
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.36981175372222

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADORA2B_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	5.595
Neutral control median absolute deviation, by plate:	0.851012
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.62730960568616
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.273962350744444
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.36981175372222

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRA1A_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_ADRA1A_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9905
Neutral control median absolute deviation, by plate:	0.202375
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.392687575678479
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.183200948036595
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.916004740182975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRA1A_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9905
Neutral control median absolute deviation, by plate:	0.202375
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.84123711753345
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.183200948036595
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.916004740182975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRA2B_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_ADRA2B_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.609
Neutral control median absolute deviation, by plate:	0.225355
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.378013483181208
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.201295979451923
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.00647989725962

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRA2B_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.803122558706695
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRB1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRB1_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRB2_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_ADRB2_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	7.122
Neutral control median absolute deviation, by plate:	1.59305
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.372298766630197
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.212432982714099
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.06216491357049

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRB2_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	7.122
Neutral control median absolute deviation, by plate:	1.59305
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.0392390998163
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	10 µM
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRB3_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_ADRB3_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.2885
Neutral control median absolute deviation, by plate:	0.515203
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.623514729857331
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.4

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.3529748038508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.764874019254

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_ADRB3_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.2885
Neutral control median absolute deviation, by plate:	0.515203
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.19392816594615
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.4

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.3529748038508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.764874019254

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_CHRM1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_CHRM1_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_CHRM3_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_CHRM3_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.64
Neutral control median absolute deviation, by plate:	0.224614
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.870505403112078
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2621797989498
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.310898994749

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_CHRM3_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.64
Neutral control median absolute deviation, by plate:	0.224614
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.811996206336538
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2621797989498
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.310898994749

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_DRD1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_DRD1_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.804
Neutral control median absolute deviation, by plate:	2.67016
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.550713171991888
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.288702945163465
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.44351472581733

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_DRD1_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.804
Neutral control median absolute deviation, by plate:	2.67016
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.29368423582205
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.288702945163465
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.44351472581733

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_DRD5_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_DRD5_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	32.4205
Neutral control median absolute deviation, by plate:	4.42185
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.440627235372944
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.258130087801469
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.29065043900734

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_DRD5_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	32.4205
Neutral control median absolute deviation, by plate:	4.42185
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.04051367127659
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.258130087801469
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.29065043900734

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_EDNRA_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_EDNRA_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.534
Neutral control median absolute deviation, by plate:	0.15938
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.04882040249769
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.361019730518374
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.80509865259187

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_EDNRA_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.534
Neutral control median absolute deviation, by plate:	0.15938
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.299693300426493
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.361019730518374
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.80509865259187

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GCGR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_GCGR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.5255
Neutral control median absolute deviation, by plate:	0.0600453
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.948406292835597
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2438076712069
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.2190383560345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GCGR_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.5255
Neutral control median absolute deviation, by plate:	0.0600453
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.84116804354865
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2438076712069
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.2190383560345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GPBAR1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_GPBAR1_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.115
Neutral control median absolute deviation, by plate:	2.93036
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.723981069561478
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.412216120387731
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	2.06108060193865

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GPBAR1_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.115
Neutral control median absolute deviation, by plate:	2.93036
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.47307235234797
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.412216120387731
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	2.06108060193865

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GPR40_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_GPR40_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9745
Neutral control median absolute deviation, by plate:	0.157156
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.460807625861694
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.16

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.228170483974698
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.14085241987349

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GPR40_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.448968029490973
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.16

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GQ_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_GQ_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.6485
Neutral control median absolute deviation, by plate:	0.360272
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.518565317142347
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.239808851609108
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.19904425804554

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GQ_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.6485
Neutral control median absolute deviation, by plate:	0.360272
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.87516068376795
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.239808851609108
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GS_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_GS_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.5525
Neutral control median absolute deviation, by plate:	0.15938
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.455582439978448
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.215532170912077
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.07766085456038

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GS_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.5525
Neutral control median absolute deviation, by plate:	0.15938
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.38100513442494
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.215532170912077
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.07766085456038

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GS1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_GS1_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.062
Neutral control median absolute deviation, by plate:	0.207564
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.409536338927578
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.230956942805705
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.15478471402853

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_GS1_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.062
Neutral control median absolute deviation, by plate:	0.207564
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.20006318745787
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.230956942805705
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.15478471402853

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HRH1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_HRH1_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.523
Neutral control median absolute deviation, by plate:	0.134175
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.35533063490715
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.219816802546314
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09908401273157

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HRH1_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.523
Neutral control median absolute deviation, by plate:	0.134175
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.53428588084685
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.219816802546314
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09908401273157

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HTR2B_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HTR2B_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HTR6_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_HTR6_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	19.1955
Neutral control median absolute deviation, by plate:	3.67018
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.693109554351313
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203810957132268
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01905478566134

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HTR6_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	19.1955
Neutral control median absolute deviation, by plate:	3.67018
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.37494122339058
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203810957132268
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01905478566134

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HTR7_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_HTR7_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.481
Neutral control median absolute deviation, by plate:	0.955536
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.448717546036637
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.156831178381937
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.784155891909685

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_HTR7_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.481
Neutral control median absolute deviation, by plate:	0.955536
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.53765062490458
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.156831178381937
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.784155891909685

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_LPAR4_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_LPAR4_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.086
Neutral control median absolute deviation, by plate:	0.201634
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.405665290389492
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.160866454981369
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.804332274906845

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_LPAR4_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.086
Neutral control median absolute deviation, by plate:	0.201634
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.866391084268117
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.160866454981369
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.804332274906845

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_06_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_06_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_19_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_19_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_32_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_32_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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References:

unspecified

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_61_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_M_61_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC1R_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_MC1R_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.3495
Neutral control median absolute deviation, by plate:	1.06154
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.522618427186252
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.261351902121235
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.30675951060617

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC1R_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.3495
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Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.28976070587685
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC2R_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_MC2R_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.796
Neutral control median absolute deviation, by plate:	0.263161
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.496878159345096
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.33

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.207234772059996
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03617386029998

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC2R_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.796
Neutral control median absolute deviation, by plate:	0.263161
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.34652644802555
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.33

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.207234772059996
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03617386029998

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC3R_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_MC3R_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.0705
Neutral control median absolute deviation, by plate:	0.191255
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.326681745086951
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.160023263908482
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.80011631954241

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC3R_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.0705
Neutral control median absolute deviation, by plate:	0.191255
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.55016189444743
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.160023263908482
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.80011631954241

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC4R_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_MC4R_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.9085
Neutral control median absolute deviation, by plate:	0.416611
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.524871647282956
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.233962619103593
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.16981309551797

Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC4R_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.9085
Neutral control median absolute deviation, by plate:	0.416611
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.00180224263399
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.233962619103593
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.16981309551797

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC5R_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_MC5R_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_PTGDR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_PTGDR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.43
Neutral control median absolute deviation, by plate:	2.12012
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.476356847567599
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.359029581887019
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.7951479094351

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_PTGDR_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.43
Neutral control median absolute deviation, by plate:	2.12012
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.88296220186195
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.359029581887019
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.7951479094351

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_PTGER2_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_PTGER2_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_PTGIR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GPCR_PTGIR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.512
Neutral control median absolute deviation, by plate:	0.180877
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.20649612316874
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.303999571186776
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.51999785593388

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GPCR_PTGIR_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.512
Neutral control median absolute deviation, by plate:	0.180877
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.29219277390189
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.303999571186776
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.51999785593388

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.582
Neutral control median absolute deviation, by plate:	0.3536
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.82371511276128
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.164730435771856
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.82365217885928

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GR_TRANS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_GR_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_GR_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR3C1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.582
Neutral control median absolute deviation, by plate:	0.3536
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.86058691678578
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.164730435771856
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.82365217885928

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_GRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.675
Neutral control median absolute deviation, by plate:	0.0770952
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.81538329581354
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0815741277707475
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.407870638853738

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_GRE_CIS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_GRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_GRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR3C1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_GRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.675
Neutral control median absolute deviation, by plate:	0.0770952
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.57621042896081
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0815741277707475
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.407870638853738

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hAR_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hAR_TRANS_XSP1_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hAR_XSP2_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hAR_XSP2_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERa_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERa_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERb_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 2 (ER beta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERb_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 2 (ER beta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERb_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 2 (ER beta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hERb_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 2 (ER beta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HIF1a_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_HIF1a_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HIF1a_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.413
Neutral control median absolute deviation, by plate:	0.0785778
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.69389687227432
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.154510376083545
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.772551880417725

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HIF1a_CIS_up

Assay Title: Attagene human HepG2 hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_HIF1a_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_HIF1a_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene HIF1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic helix-loop-helix protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HIF1a_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.413
Neutral control median absolute deviation, by plate:	0.0785778
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.3423171649041
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.154510376083545
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.772551880417725

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HNF4a_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_HNF4a_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HNF4a_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.989
Neutral control median absolute deviation, by plate:	1.33212
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.4757334309664
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.45

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.288413542799086
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.44206771399543

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HNF4a_TRANS_up

Assay Title: Attagene human HepG2 hepatocyte nuclear factor 4, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_HNF4a_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_HNF4a_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene HNF4A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "orphan".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HNF4a_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.989
Neutral control median absolute deviation, by plate:	1.33212
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.77338738156823
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.45

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.288413542799086
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.44206771399543

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HNF4g_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_HNF4g_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.5545
Neutral control median absolute deviation, by plate:	0.453676
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.01681548381309
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.29

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.368727139296372
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.84363569648186

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HNF4g_TRANS2_up

Assay Title: Attagene human HepG2 hepatocyte nuclear factor 4, gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.5545
Neutral control median absolute deviation, by plate:	0.453676
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.867818274293011
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.29

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	10 µM
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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HNF6_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_HNF6_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HNF6_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.722
Neutral control median absolute deviation, by plate:	0.0533736
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.80686412598521
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0725342005615792
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.362671002807896

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HNF6_CIS_up

Assay Title: Attagene human HepG2 one cut homeobox 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_HNF6_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_HNF6_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene ONECUT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "homeobox protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HNF6_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.722
Neutral control median absolute deviation, by plate:	0.0533736
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.36183196820815
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0725342005615792
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.362671002807896

Chemical Library Scope and Limitations:

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availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

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EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Hpa5_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Hpa5_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Hpa5_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.892860627457303
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.166751257648282
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.83375628824141

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Hpa5_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Hpa5_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Hpa5_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Hpa5_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.88970471319602
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.166751257648282
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.83375628824141

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hPPARg_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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1.1 Overview

Assay Summary:

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1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
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Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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ATG_hPPARg_XSP2_dn

Assay Title: Attagene human HepG2 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
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Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hPPARg_XSP2_up

Assay Title: Attagene human HepG2 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HSE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_HSE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HSE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.595
Neutral control median absolute deviation, by plate:	0.0622692
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.83935743792305
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0952832777912402
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.476416388956201

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_HSE_CIS_up

Assay Title: Attagene human HepG2 heat shock transcription factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_HSE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_HSE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene HSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "heat shock protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_HSE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.595
Neutral control median absolute deviation, by plate:	0.0622692
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.95595141405941
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0952832777912402
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.476416388956201

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRa_XSP2_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRa_XSP2_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

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MIE, Molecular Initiating Event

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SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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ATG_hTRb_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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3.2 Assay Performance

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Target (nominal) number of replicates:	unspecified
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRb_TRANS_XSP1_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRb_XSP2_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_hTRb_XSP2_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_IR1_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_IR1_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_IR1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.633
Neutral control median absolute deviation, by plate:	0.155673
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.61470984411521
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.25

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0976059561665389
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.488029780832694

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ISRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_ISRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_ISRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.325
Neutral control median absolute deviation, by plate:	0.0978516
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.43217341159287
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.141145183019864
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.70572591509932

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_ISRE_CIS_up

Assay Title: Attagene human HepG2 interferon regulatory factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_ISRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_ISRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene IRF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "interferon regulatory factors".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_ISRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.325
Neutral control median absolute deviation, by plate:	0.0978516
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.75133463501744
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.141145183019864
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.70572591509932

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_LRH1_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_LRH1_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.6915
Neutral control median absolute deviation, by plate:	0.517427
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.787998176168295
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.33179847144245
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.65899235721225

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_LRH1_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 5, group A, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.6915
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Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.944085220026855
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_LXRa_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_LXRa_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_LXRa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.5375
Neutral control median absolute deviation, by plate:	0.343963
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.12555278872999
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.230111357105154
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.15055678552577

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_LXRa_TRANS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group H, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_LXRa_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_LXRa_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1H3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_LXRa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.5375
Neutral control median absolute deviation, by plate:	0.343963
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.78483131216003
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.230111357105154
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.15055678552577

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_LXRb_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_LXRb_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_LXRb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.003
Neutral control median absolute deviation, by plate:	0.278729
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.63538633154773
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.28

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203957964171857
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01978982085929

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_LXRb_TRANS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group H, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_LXRb_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_LXRb_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1H2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

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Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

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Caveats:

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Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.28

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Biological Response:

unspecified

Analytical Elements:

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Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203957964171857
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01978982085929

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_06_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_06_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_06_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.045
Neutral control median absolute deviation, by plate:	0.0266868
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.532910367892415
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.023982430007003
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_06_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_06_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_06_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_06_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.045
Neutral control median absolute deviation, by plate:	0.0266868
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.544771428882858
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.023982430007003
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_06_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_06_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_06_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9935
Neutral control median absolute deviation, by plate:	0.0318759
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.557011360434053
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0358169635014388
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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NCI, Normalized Cell Index

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_06_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_06_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_06_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_06_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9935
Neutral control median absolute deviation, by plate:	0.0318759
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.57007165306087
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0358169635014388
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_19_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_19_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_19_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.91
Neutral control median absolute deviation, by plate:	0.051891
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.58236538923512
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0554428766521351
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.277214383260675

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_19_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_19_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_19_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_19_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.91
Neutral control median absolute deviation, by plate:	0.051891
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.05602138296864
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0554428766521351
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.277214383260675

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_19_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_19_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_19_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.013
Neutral control median absolute deviation, by plate:	0.0637518
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.53453119933638
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0719687013207205
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.359843506603603

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_19_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_19_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_19_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_19_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.013
Neutral control median absolute deviation, by plate:	0.0637518
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.727607196510084
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0719687013207205
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.359843506603603

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_32_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_32_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_32_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.874
Neutral control median absolute deviation, by plate:	0.223873
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.51006118942537
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0533510676488249
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.266755338244125

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_32_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_32_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_32_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_32_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.874
Neutral control median absolute deviation, by plate:	0.223873
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.96790637029015
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0533510676488249
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.266755338244125

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_32_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_32_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_32_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4.1925
Neutral control median absolute deviation, by plate:	0.260196
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.11332456375228
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0668644660299623
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.334322330149811

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_32_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_32_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_32_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_32_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4.1925
Neutral control median absolute deviation, by plate:	0.260196
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.07707204324486
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0668644660299623
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.334322330149811

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_61_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_61_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_61_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.045
Neutral control median absolute deviation, by plate:	0.0266868
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.532910367892415
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0239747516257868
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_61_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_61_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_61_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_61_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.045
Neutral control median absolute deviation, by plate:	0.0266868
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.544771428882858
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0239747516257868
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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UGT, UDP-glucuronosyltransferase

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U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_61_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_M_61_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_61_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.994
Neutral control median absolute deviation, by plate:	0.0326172
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.557011360434053
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0358169635014388
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.263034405833794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M_61_TRANS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_M_61_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_M_61_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_M_61_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	0.0326172
Positive control well median response value, by plate:	unspecified
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Negative control well median, by plate:	0.580281344603248
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
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Chemical Library Scope and Limitations:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M06_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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ATG_M06_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Positive control well median response value, by plate:	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M06_XSP2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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ATG_M06_XSP2_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

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1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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ATG_M19_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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5. Potential Regulatory Applications

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ATG_M19_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
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Assay Title: Attagene human HepG2 unspecified assay

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unspecified

1.2 Assay Definition

Assay Throughput:

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Experimental System:

adherent HepG2 cell line

Basic Procedure:
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Proprietary Elements:

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Caveats:

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2. Assay Component Descriptions

Assay Objectives:

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Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M19_XSP2_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M32_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M32_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M32_XSP2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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Assay Source Information:

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References:

unspecified

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RTCA, Real-Time Cell Analysis

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_M32_XSP2_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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ATG_M61_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

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Assay Summary:

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Experimental System:

adherent HepG2 cell line

Basic Procedure:
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Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
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Positive control well median absolute deviation, by plate:	unspecified
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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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ATG_M61_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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ATG_M61_XSP2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
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CV (median across all plates):	unspecified

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3.1 Data Interpretation

Biological Response:

unspecified

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unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPPARg_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 peroxisome proliferator activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator activated receptor gamma [Â Mus musculus(house mouse)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPPARg_TRANS_XSP1_up

Assay Title: Attagene human HepG2 peroxisome proliferator activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator activated receptor gamma [Â Mus musculus(house mouse)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPPARg_XSP2_dn

Assay Title: Attagene human HepG2 peroxisome proliferator activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator activated receptor gamma [Â Mus musculus(house mouse)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

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ATG_mPPARg_XSP2_up

Assay Title: Attagene human HepG2 peroxisome proliferator activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

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Analytical Elements:

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3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPXR_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPXR_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPXR_XSP2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_mPXR_XSP2_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_MR_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_MR_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.8505
Neutral control median absolute deviation, by plate:	0.0978516
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.422279243767098
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.163904191615211
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.819520958076055

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_MR_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 3, group C, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.8505
Neutral control median absolute deviation, by plate:	0.0978516
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.50029217673417
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.163904191615211
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.819520958076055

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_MRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_MRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_MRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.529
Neutral control median absolute deviation, by plate:	0.10823
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.11638657455114
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.120295090866866
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.60147545433433

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_MRE_CIS_up

Assay Title: Attagene human HepG2 metal-regulatory transcription factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_MRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_MRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene MTF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "zinc finger".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_MRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.529
Neutral control median absolute deviation, by plate:	0.10823
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.68409148514951
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.120295090866866
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.60147545433433

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Myb_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Myb_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Myb_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.907
Neutral control median absolute deviation, by plate:	0.0474432
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.18966690530377
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0595512425916688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.297756212958344

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Myb_CIS_up

Assay Title: Attagene human HepG2 v-myb avian myeloblastosis viral oncogene homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Myb_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Myb_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene MYB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "MYB proteins".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Myb_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.907
Neutral control median absolute deviation, by plate:	0.0474432
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.11941516694477
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0595512425916688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.297756212958344

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Myc_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Myc_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Myc_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.032
Neutral control median absolute deviation, by plate:	0.155673
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.60760430388259
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0986758598304688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.493379299152344

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Myc_CIS_up

Assay Title: Attagene human HepG2 v-myc avian myelocytomatosis viral oncogene homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Myc_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Myc_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene MYC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic helix-loop-helix leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Myc_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.032
Neutral control median absolute deviation, by plate:	0.155673
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.46949449376648
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0986758598304688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.493379299152344

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NF_kB_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NF_kB_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NF_kB_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.545
Neutral control median absolute deviation, by plate:	0.179395
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.96646304028077
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.33

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.12812584744916
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.6406292372458

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NF_kB_CIS_up

Assay Title: Attagene human HepG2 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_NF_kB_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_NF_kB_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NFKB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "NF-kappa B".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NF_kB_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.545
Neutral control median absolute deviation, by plate:	0.179395
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.66098283465807
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.33

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.12812584744916
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.6406292372458

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NFI_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NFI_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NFI_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.878
Neutral control median absolute deviation, by plate:	0.146777
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.80527160755711
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.17

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0837715095541358
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.418857547770679

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

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4. Assay Documentation

4.1 Resources

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NFI_CIS_up

Assay Title: Attagene human HepG2 nuclear factor I/A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_NFI_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_NFI_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NFIA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "nuclear factor I".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NFI_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.878
Neutral control median absolute deviation, by plate:	0.146777
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.25359213570062
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.17

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0837715095541358
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.418857547770679

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NOR1_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NOR1_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.0825
Neutral control median absolute deviation, by plate:	0.206823
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.345365534299684
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.177303501697702
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.88651750848851

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NOR1_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 4, group A, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.0825
Neutral control median absolute deviation, by plate:	0.206823
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.11254705943087
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.177303501697702
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.88651750848851

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NRF1_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NRF1_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NRF1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.745
Neutral control median absolute deviation, by plate:	0.0978516
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.82093663450045
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0798138850312679
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.39906942515634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NRF1_CIS_up

Assay Title: Attagene human HepG2 nuclear respiratory factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_NRF1_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_NRF1_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NRF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "nuclear respiratory factors".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NRF1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.745
Neutral control median absolute deviation, by plate:	0.0978516
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.53986266543566
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0798138850312679
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.39906942515634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NRF2_ARE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NRF2_ARE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NRF2_ARE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.242
Neutral control median absolute deviation, by plate:	0.0637518
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.60050764534579
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.15619694777724
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.7809847388862

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NRF2_ARE_CIS_up

Assay Title: Attagene human HepG2 nuclear factor, erythroid 2-like 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_NRF2_ARE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_NRF2_ARE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NFE2L2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NRF2_ARE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.242
Neutral control median absolute deviation, by plate:	0.0637518
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.22032374441061
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.15619694777724
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.7809847388862

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NUR77_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NUR77_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.013
Neutral control median absolute deviation, by plate:	0.0756126
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.320133002375977
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.171893199330648
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.85946599665324

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NUR77_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 4, group A, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.013
Neutral control median absolute deviation, by plate:	0.0756126
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.437691508838884
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.171893199330648
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.85946599665324

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NURR1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_NURR1_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NURR1_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4.5055
Neutral control median absolute deviation, by plate:	1.69091
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.46511525714088
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.38

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.175286741331947
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.876433706659735

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_NURR1_TRANS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 4, group A, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_NURR1_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_NURR1_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR4A2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "orphan".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_NURR1_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4.5055
Neutral control median absolute deviation, by plate:	1.69091
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.41588757388508
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.38

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.175286741331947
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.876433706659735

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Oct_MLP_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Oct_MLP_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Oct_MLP_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.73
Neutral control median absolute deviation, by plate:	0.346928
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.35338745514436
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.139861446762469
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.699307233812345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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FBS, Fetal Bovine Serum

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Oct_MLP_CIS_up

Assay Title: Attagene human HepG2 POU class 2 homeobox 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Oct_MLP_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Oct_MLP_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene POU2F1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "POU domain protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Oct_MLP_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.73
Neutral control median absolute deviation, by plate:	0.346928
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.22233346724532
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.139861446762469
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.699307233812345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_p53_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_p53_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_p53_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.426
Neutral control median absolute deviation, by plate:	0.157156
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.82017896241519
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.37

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1281857235595
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.6409286177975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_p53_CIS_up

Assay Title: Attagene human HepG2 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_p53_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_p53_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "tumor suppressor".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_p53_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.426
Neutral control median absolute deviation, by plate:	0.157156
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.12845838414693
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.37

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1281857235595
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.6409286177975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Pax6_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Pax6_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Pax6_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.473
Neutral control median absolute deviation, by plate:	0.0874734
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.98125703157583
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.124647117944891
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.623235589724455

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Pax6_CIS_up

Assay Title: Attagene human HepG2 paired box 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Pax6_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Pax6_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene PAX6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "paired box protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Pax6_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.473
Neutral control median absolute deviation, by plate:	0.0874734
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.39466483035971
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.124647117944891
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.623235589724455

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PBREM_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PBREM_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PBREM_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.517
Neutral control median absolute deviation, by plate:	0.0696822
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.18322182405577
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0972702661106279
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.48635133055314

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PBREM_CIS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group I, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_PBREM_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_PBREM_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1I3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PBREM_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.517
Neutral control median absolute deviation, by plate:	0.0696822
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.87752613897408
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0972702661106279
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.48635133055314

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PNR_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PNR_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.552
Neutral control median absolute deviation, by plate:	0.0704235
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.877132917280296
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.265245674443195
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.32622837221598

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PNR_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group E, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.552
Neutral control median absolute deviation, by plate:	0.0704235
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.446183533436449
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.265245674443195
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.32622837221598

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PPARa_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PPARa_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PPARa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.455
Neutral control median absolute deviation, by plate:	1.33434
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.10697100013955
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.39

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.233973966546105
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.16986983273053

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PPARd_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PPARd_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PPARd_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.7805
Neutral control median absolute deviation, by plate:	0.337291
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.50514991894313
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.43

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.224567659937351
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.12283829968675

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PPARg_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PPARg_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PPARg_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.6625
Neutral control median absolute deviation, by plate:	1.08452
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.65056222720786
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.41

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.234827250783085
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17413625391542

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PPRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PPRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PPRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.26
Neutral control median absolute deviation, by plate:	0.62714
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.58363028437608
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.5

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.180050885490761
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.900254427453805

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PR_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PR_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9175
Neutral control median absolute deviation, by plate:	0.123056
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.239168483413164
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.145431246931568
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.72715623465784

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PR_TRANS2_up

Assay Title: Attagene human HepG2 progesterone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9175
Neutral control median absolute deviation, by plate:	0.123056
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.69760576323559
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.145431246931568
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.72715623465784

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PXR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PXR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PXR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.481
Neutral control median absolute deviation, by plate:	0.523358
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.20608407428997
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.263684293832972
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.31842146916486

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PXR_TRANS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group I, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_PXR_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_PXR_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1I2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PXR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.481
Neutral control median absolute deviation, by plate:	0.523358
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.20716242584662
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.263684293832972
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.31842146916486

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PXRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_PXRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PXRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.478
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.39726852596715
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.133814814954859
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.669074074774295

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_PXRE_CIS_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group I, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_PXRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_PXRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene NR1I2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_PXRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.478
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.97146339387879
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.133814814954859
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.669074074774295

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AOP, Adverse Outcome Pathway

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RARa_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RARa_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RARa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.008
Neutral control median absolute deviation, by plate:	5.29585
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.11984563379082
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.59

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.230829613173288
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.15414806586644

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RARa_TRANS_up

Assay Title: Attagene human HepG2 retinoic acid receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RARa_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RARa_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RARA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RARa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9.008
Neutral control median absolute deviation, by plate:	5.29585
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.52068626802098
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.59

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.230829613173288
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.15414806586644

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RARb_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RARb_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RARb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5.2965
Neutral control median absolute deviation, by plate:	2.44258
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.9540504141395
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.46

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.219203288540305
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09601644270152

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RARb_TRANS_up

Assay Title: Attagene human HepG2 retinoic acid receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RARb_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RARb_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RARB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RARb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5.2965
Neutral control median absolute deviation, by plate:	2.44258
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.24153514179572
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.46

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.219203288540305
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.09601644270152

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RARg_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RARg_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RARg_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.252
Neutral control median absolute deviation, by plate:	2.72873
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.71274880891838
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.44

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.234752620008944
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17376310004472

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RARg_TRANS_up

Assay Title: Attagene human HepG2 retinoic acid receptor, gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RARg_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RARg_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RARG. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RARg_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.252
Neutral control median absolute deviation, by plate:	2.72873
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.32843293183805
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.44

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.234752620008944
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.17376310004472

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Rev_ERB_A_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Rev_ERB_A_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.8285
Neutral control median absolute deviation, by plate:	0.0615279
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.391595647506572
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.18287907675056
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.9143953837528

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Rev_ERB_A_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group D, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.8285
Neutral control median absolute deviation, by plate:	0.0615279
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.299410001444411
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.18287907675056
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.9143953837528

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Rev_ERB_B_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Rev_ERB_B_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.642
Neutral control median absolute deviation, by plate:	0.066717
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.740577351168642
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.205785264711313
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.02892632355657

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Rev_ERB_B_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 1, group D, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.642
Neutral control median absolute deviation, by plate:	0.066717
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.461602856797519
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.205785264711313
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.02892632355657

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORa_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RORa_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.3625
Neutral control median absolute deviation, by plate:	0.198668
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.358477478314915
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.264862770532252
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.32431385266126

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORa_TRANS2_up

Assay Title: Attagene human HepG2 RAR-related orphan receptor A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.3625
Neutral control median absolute deviation, by plate:	0.198668
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.851510276891427
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.264862770532252
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.32431385266126

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORb_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RORb_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RORb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.9055
Neutral control median absolute deviation, by plate:	1.95407
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.29581186627866
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.5

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.330503763155624
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.65251881577812

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORb_TRANS_up

Assay Title: Attagene human HepG2 RAR-related orphan receptor B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RORb_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RORb_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RORB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "orphan".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RORb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.9055
Neutral control median absolute deviation, by plate:	1.95407
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.9547535506568
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.5

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.330503763155624
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.65251881577812

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RORE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RORE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.367
Neutral control median absolute deviation, by plate:	0.0934038
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.94587478613903
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.25

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.139162339586152
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.69581169793076

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORE_CIS_up

Assay Title: Attagene human HepG2 RAR-related orphan receptor A|RAR-related orphan receptor B|RAR-related orphan receptor C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RORE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RORE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RORA and RORB and RORC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "orphan".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RORE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.367
Neutral control median absolute deviation, by plate:	0.0934038
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.37063340550095
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.25

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.139162339586152
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.69581169793076

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORg_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RORg_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RORg_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.965
Neutral control median absolute deviation, by plate:	0.623433
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.46964181723952
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.236150597083625
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.18075298541813

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RORg_TRANS_up

Assay Title: Attagene human HepG2 RAR-related orphan receptor C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RORg_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RORg_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RORC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "orphan".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RORg_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.965
Neutral control median absolute deviation, by plate:	0.623433
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.82929918858663
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.236150597083625
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.18075298541813

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RXRa_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RXRa_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RXRa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.0905
Neutral control median absolute deviation, by plate:	0.346928
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.21489048518087
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.186160984681131
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.930804923405655

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RXRa_TRANS_up

Assay Title: Attagene human HepG2 retinoid X receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RXRa_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RXRa_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RXRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RXRa_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.0905
Neutral control median absolute deviation, by plate:	0.346928
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.49776114360993
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.186160984681131
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.930804923405655

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RXRb_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RXRb_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RXRb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.0105
Neutral control median absolute deviation, by plate:	0.695339
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.43382834917291
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.202526652337239
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.0126332616862

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RXRb_TRANS_up

Assay Title: Attagene human HepG2 retinoid X receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_RXRb_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_RXRb_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene RXRB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_RXRb_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.0105
Neutral control median absolute deviation, by plate:	0.695339
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.27108722360708
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.202526652337239
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.0126332616862

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RXRg_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_RXRg_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.872
Neutral control median absolute deviation, by plate:	0.081543
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.440317993246176
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203095463566057
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01547731783028

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_RXRg_TRANS2_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.872
Neutral control median absolute deviation, by plate:	0.081543
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.74049587418707
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203095463566057
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.01547731783028

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_SF_1_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_SF_1_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.4995
Neutral control median absolute deviation, by plate:	1.05042
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.773623685419073
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.360364549742844
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.80182274871422

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_SF_1_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 5, group A, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.4995
Neutral control median absolute deviation, by plate:	1.05042
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.17194372821419
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.360364549742844
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.80182274871422

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_SHP_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_SHP_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.6645
Neutral control median absolute deviation, by plate:	0.0481845
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.591851701946887
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.193771718433799
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.968858592168995

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_SHP_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 0, group B, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.6645
Neutral control median absolute deviation, by plate:	0.0481845
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.488919677644374
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.193771718433799
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.968858592168995

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Sox_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Sox_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Sox_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.774
Neutral control median absolute deviation, by plate:	0.0800604
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.05388556833575
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0882130309123666
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.441065154561833

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Sox_CIS_up

Assay Title: Attagene human HepG2 SRY (sex determining region Y)-box 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Sox_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Sox_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene SOX1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "HMG box protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Sox_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.774
Neutral control median absolute deviation, by plate:	0.0800604
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.61216381515812
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0882130309123666
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.441065154561833

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Sp1_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Sp1_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Sp1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.572
Neutral control median absolute deviation, by plate:	0.117125
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.65105169117893
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.10533198120005
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.52665990600025

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AOP, Adverse Outcome Pathway

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DMSO, Dimethyl Sulfoxide

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Sp1_CIS_up

Assay Title: Attagene human HepG2 Sp1 transcription factor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Sp1_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Sp1_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene SP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "zinc finger".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Sp1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.572
Neutral control median absolute deviation, by plate:	0.117125
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.85535190108196
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.10533198120005
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.52665990600025

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_SREBP_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_SREBP_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_SREBP_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.984
Neutral control median absolute deviation, by plate:	0.217942
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.95951083527303
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0727012976619559
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.363506488309779

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_SREBP_CIS_up

Assay Title: Attagene human HepG2 sterol regulatory element binding transcription factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_SREBP_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_SREBP_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene SREBF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic helix-loop-helix leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_SREBP_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.984
Neutral control median absolute deviation, by plate:	0.217942
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.80689534306538
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0727012976619559
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.363506488309779

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_STAT3_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_STAT3_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_STAT3_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.619
Neutral control median absolute deviation, by plate:	0.0400302
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.94180775225555
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.087781538771495
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.438907693857475

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_STAT3_CIS_up

Assay Title: Attagene human HepG2 signal transducer and activator of transcription 3 (acute-phase response factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_STAT3_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_STAT3_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene STAT3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "stat protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_STAT3_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.619
Neutral control median absolute deviation, by plate:	0.0400302
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.91995625348721
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.087781538771495
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.438907693857475

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TA_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TA_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TA_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.282
Neutral control median absolute deviation, by plate:	0.0607866
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.07445899220045
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.147321134276289
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.736605671381445

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TA_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_TA_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_TA_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TA_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.282
Neutral control median absolute deviation, by plate:	0.0607866
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.38392742004238
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.147321134276289
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.1 Resources

Assay Source Information:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TAL_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TAL_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TAL_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.208
Neutral control median absolute deviation, by plate:	0.0400302
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.32192809488736
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.138581273826894
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.69290636913447

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TAL_CIS_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_TAL_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_TAL_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the background control at the transcription factor-level as they relate to the gene . Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "background measurement" intended target family, where the subfamily is "internal marker".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TAL_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.208
Neutral control median absolute deviation, by plate:	0.0400302
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.75974205483166
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.138581273826894
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.69290636913447

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TCF_b_cat_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TCF_b_cat_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TCF_b_cat_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.39
Neutral control median absolute deviation, by plate:	0.219425
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.668472750346
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.56

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.139299232619389
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.696496163096945

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TCF_b_cat_CIS_up

Assay Title: Attagene human HepG2 transcription factor 7 (T-cell specific, HMG-box)|transcription factor 7-like 2 (T-cell specific, HMG-box)|lymphoid enhancer-binding factor 1|transcription factor 7-like 1 (T-cell specific, HMG-box) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_TCF_b_cat_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_TCF_b_cat_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene TCF7 and TCF7L2 and LEF1 and TCF7L1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "HMG box protein".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TCF_b_cat_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long

term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.39
Neutral control median absolute deviation, by plate:	0.219425
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.98154309529244
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.56

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA|NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.139299232619389
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.696496163096945

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TGFb_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TGFb_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TGFb_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.265
Neutral control median absolute deviation, by plate:	0.0474432
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.65105169117893
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.157432164655408
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.78716082327704

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TGFb_CIS_up

Assay Title: Attagene human HepG2 transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_TGFb_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_TGFb_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene TGFB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "growth factor" intended target family, where the subfamily is "transforming growth factor beta".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_TGFb_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.265
Neutral control median absolute deviation, by plate:	0.0474432
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.05990302825098
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.157432164655408
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.78716082327704

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_THRa1_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_THRa1_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_THRa1_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.9215
Neutral control median absolute deviation, by plate:	0.24537
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.53302228530608
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.225363774990836
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.12681887495418

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_THRb_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_THRb_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.303
Neutral control median absolute deviation, by plate:	0.0385476
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.77868905587251
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.278016730457477
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.39008365228738

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TLX_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TLX_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.5225
Neutral control median absolute deviation, by plate:	0.0956277
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.13133486930972
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.247030231434165
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.23515115717082

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TLX_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group E, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.5225
Neutral control median absolute deviation, by plate:	0.0956277
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.288123697118213
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.247030231434165
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.23515115717082

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TR2_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TR2_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.958
Neutral control median absolute deviation, by plate:	0.0934038
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.33443347959581
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.179476629256447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.897383146282235

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TR2_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group C, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.958
Neutral control median absolute deviation, by plate:	0.0934038
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.324842147956951
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
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Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.179476629256447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.897383146282235

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TR4_TRANS2_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_TR4_TRANS2_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.7485
Neutral control median absolute deviation, by plate:	0.119349
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.444267007967424
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.16

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.200221152576159
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.00110576288079

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_TR4_TRANS2_up

Assay Title: Attagene human HepG2 nuclear receptor subfamily 2, group C, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.7485
Neutral control median absolute deviation, by plate:	0.119349
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.441737366829335
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.16

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0399999999999998 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.200221152576159
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.00110576288079

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trAR_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

PREDICTED: Chrysemys picta bellii androgen receptor (AR), mRNA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trAR_TRANS_XSP1_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

PREDICTED: Chrysemys picta bellii androgen receptor (AR), mRNA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trAR_XSP2_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

PREDICTED: Chrysemys picta bellii androgen receptor (AR), mRNA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trAR_XSP2_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

PREDICTED: Chrysemys picta bellii androgen receptor (AR), mRNA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trERa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trERa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trERa_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trERa_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 1 [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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DMSO, Dimethyl Sulfoxide

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MIE, Molecular Initiating Event

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trTRa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trTRa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_trTRa_XSP2_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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SSMD, Strictly Standardized Mean Difference

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ATG_trTRa_XSP2_up

Assay Title: Attagene human HepG2 thyroid hormone receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor, alpha [Â Chrysemys pictaÂ (painted turtle)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_VDR_TRANS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_VDR_TRANS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_VDR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.961
Neutral control median absolute deviation, by plate:	0.260938
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.19446551356676
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.173479057381292
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.86739528690646

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_VDR_TRANS_up

Assay Title: Attagene human HepG2 vitamin D (1,25- dihydroxyvitamin D3) receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_VDR_TRANS was analyzed into 1 assay endpoint. This assay endpoint, ATG_VDR_TRANS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene VDR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_VDR_TRANS, is one of 30 assay component(s) measured or calculated from the ATG_TRANS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_TRANS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.961
Neutral control median absolute deviation, by plate:	0.260938
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.64252829941384
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.173479057381292
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.86739528690646

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_VDRE_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_VDRE_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_VDRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.546
Neutral control median absolute deviation, by plate:	0.126021
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.34340140234484
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.145673531648076
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.72836765824038

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

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DMSO, Dimethyl Sulfoxide

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_VDRE_CIS_up

Assay Title: Attagene human HepG2 vitamin D (1,25- dihydroxyvitamin D3) receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_VDRE_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_VDRE_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene VDR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_VDRE_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.546
Neutral control median absolute deviation, by plate:	0.126021
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.74019742737882
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.145673531648076
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.72836765824038

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Xbp1_CIS_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

ATG_Xbp1_CIS_dn was not developed or optimized to detect loss of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Xbp1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.729
Neutral control median absolute deviation, by plate:	0.225355
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.7520511397034
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.11881457365277
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.59407286826385

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_Xbp1_CIS_up

Assay Title: Attagene human HepG2 X-box binding protein 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_Xbp1_CIS was analyzed into 1 assay endpoint. This assay endpoint, ATG_Xbp1_CIS_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the gene XBP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "dna binding" intended target family, where the subfamily is "basic leucine zipper".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_Xbp1_CIS, is one of 52 assay component(s) measured or calculated from the ATG_CIS assay. It is designed to make measurements of mRNA induction, a form of inducible reporter, as detected with fluorescence intensity signals by Reverse transcription polymerase chain reaction (RT-PCR) and Capillary electrophoresis technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_CIS is a cell-based, multiplexed-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.729
Neutral control median absolute deviation, by plate:	0.225355
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.51127788896987
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	7
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.11881457365277
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.59407286826385

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_XTT_Cytotoxicity_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Not intended signal direction and should be used with caution

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_XTT_Cytotoxicity, is one of 1 assay component(s) measured or calculated from the ATG_XTT_Cytotoxicity assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by XTT cytotoxicity assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_XTT_Cytotoxicity is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is

considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_XTT_Cytotoxicity_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_XTT_Cytotoxicity_TRANS_XSP1_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_XTT_Cytotoxicity_up

Assay Title: Attagene human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component ATG_XTT_Cytotoxicity was analyzed into 1 assay endpoint. This assay endpoint, ATG_XTT_Cytotoxicity_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, loss-of-signal activity can be used to understand changes in the viability. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
ATG_XTT_Cytotoxicity, is one of 1 assay component(s) measured or calculated from the ATG_XTT_Cytotoxicity assay. It is designed to make measurements of cell number, a form of viability reporter, as detected with fluorescence intensity signals by XTT cytotoxicity assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

ATG_XTT_Cytotoxicity is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 24-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	97.9175238663782
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.8 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	14.9837525813246
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	74.918762906623

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

Romanov S, Medvedev A, Gambarian M, Poltoratskaya N, Moeser M, Medvedeva L, Gambarian M, Diatchenko L, Makarov S. Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. Nat Methods. 2008 Mar;5(3):253-60. doi: 10.1038/nmeth.1186. Epub 2008 Feb 24. PubMed PMID: 18297081.|Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem Res Toxicol. 2010 Mar 15;23(3):578-90. doi: 10.1021/tx900325g. PubMed PMID: 20143881.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfAR_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfAR_TRANS_XSP1_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfAR_XSP2_dn

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfAR_XSP2_up

Assay Title: Attagene human HepG2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

androgen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER1_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER1_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER1_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER1_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor [Danio rerio (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2a_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 2a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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EDC, Endocrine disrupting chemicals

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Contact Information:

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5. Potential Regulatory Applications

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ATG_zfER2a_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 2a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2a_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 2a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2a_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 2a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2b_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 estrogen receptor 2b assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2b [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2b_TRANS_XSP1_up

Assay Title: Attagene human HepG2 estrogen receptor 2b assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2b [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

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References:

unspecified

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2b_XSP2_dn

Assay Title: Attagene human HepG2 estrogen receptor 2b assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2b [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfER2b_XSP2_up

Assay Title: Attagene human HepG2 estrogen receptor 2b assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

estrogen receptor 2b [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfPPARg_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator-activated receptor gamma [Â Danio rerio(zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfPPARg_TRANS_XSP1_up

Assay Title: Attagene human HepG2 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator-activated receptor gamma [Â Danio rerio(zebrafish)]

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

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References:

unspecified

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Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

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Caveats:

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Status:

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Method Updates / Confirmatory Studies:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator-activated receptor gamma [Â Danio rerio(zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfPPARg_XSP2_up

Assay Title: Attagene human HepG2 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

peroxisome proliferator-activated receptor gamma [Â Danio rerio(zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRa_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor alpha a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor alpha a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRa_TRANS_XSP1_up

Assay Title: Attagene human HepG2 thyroid hormone receptor alpha a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor alpha a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRa_XSP2_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor alpha a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor alpha a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRa_XSP2_up

Assay Title: Attagene human HepG2 thyroid hormone receptor alpha a assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor alpha a [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRb_TRANS_XSP1_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor beta [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

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4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRb_TRANS_XSP1_up

Assay Title: Attagene human HepG2 thyroid hormone receptor beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor beta [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

ATG_zfTRb_XSP2_dn

Assay Title: Attagene human HepG2 thyroid hormone receptor beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor beta [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

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unspecified

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ATG_zfTRb_XSP2_up

Assay Title: Attagene human HepG2 thyroid hormone receptor beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid hormone receptor beta [Â Danio rerioÂ (zebrafish)]

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

XTT_Cytotoxicity_XSP2_dn

Assay Title: XTT_Cytotoxicity_XSP2_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

XTT_Cytotoxicity_XSP2_up

Assay Title: XTT_Cytotoxicity_XSP2_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

24-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Attagene Inc. is a CRO offering a unique screening service using its proprietary multiplexed pathway profiling platform, the FACTORIAL.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BRL_CHO_hypodiploid_freq_up

Assay Title: BioReliance Corporation chinese hamster CHO unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent CHO cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BRL_CHO_micronucleus_freq_up

Assay Title: BioReliance Corporation chinese hamster CHO unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent CHO cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BRL_CHO_relative_survival_dn

Assay Title: BioReliance Corporation chinese hamster CHO unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent CHO cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BRL_CHO_S9_hypodiploid_freq_up

Assay Title: BioReliance Corporation chinese hamster CHO unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent CHO cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BRL_CHO_S9_micronucleus_freq_up

Assay Title: BioReliance Corporation chinese hamster CHO unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent CHO cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BRL_CHO_S9_relative_survival_dn

Assay Title: BioReliance Corporation chinese hamster CHO unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent CHO cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Eselectin_down

Assay Title: Bioseek human umbilical vein endothelium selectin E assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Eselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Eselectin_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Eselectin, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.48761475
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02294621888076
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Eselectin_up

Assay Title: Bioseek human umbilical vein endothelium selectin E assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Eselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Eselectin_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Eselectin, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.10461025
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02294621888076
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_HLADR_down

Assay Title: Bioseek human umbilical vein endothelium major histocompatibility complex, class II, DR alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_HLADR was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_HLADR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene HLA-DRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "MHC Class II".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_HLADR, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.34043955
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0276864645477
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0830593936431

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_HLADR_up

Assay Title: Bioseek human umbilical vein endothelium major histocompatibility complex, class II, DR alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_HLADR was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_HLADR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene HLA-DRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "MHC Class II".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_HLADR, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.19388486
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Chemical Library Scope and Limitations:

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availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_ICAM1_down

Assay Title: Bioseek human umbilical vein endothelium intercellular adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_ICAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_ICAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene ICAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_ICAM1, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.47437305
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.016993160898
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_ICAM1_up

Assay Title: Bioseek human umbilical vein endothelium intercellular adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_ICAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_ICAM1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene ICAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_ICAM1, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.080123514
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.016993160898
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_IL8_down

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_IL8_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_IL8, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3473747
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Nominal number of tested concentrations:	4
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Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0338699058201
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1016097174603

Chemical Library Scope and Limitations:

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_IL8_up

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_IL8_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_IL8, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.19429884
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0338699058201
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1016097174603

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_MCP1_down

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_MCP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_MCP1, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3863223
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0301114481031
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0903343443093

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_MCP1_up

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_MCP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_MCP1, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.25647965
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0301114481031
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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_MIG_down

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_MIG_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_MIG, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.7664985
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00650651411949
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_MIG_up

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_MIG_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_MIG, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.04251108
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00650651411949
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Proliferation_down

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Proliferation_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Proliferation, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.51436955
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0378282506343
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1134847519029

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Proliferation_up

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Proliferation_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Proliferation, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.156311055
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0378282506343
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1134847519029

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_SRB_down

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_SRB, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.20364755
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0239123016489
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_SRB_up

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_SRB, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.124
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0239123016489
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Thrombomodulin_down

Assay Title: Bioseek human umbilical vein endothelium thrombomodulin assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Thrombomodulin was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Thrombomodulin_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene THBD. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Thrombomodulin, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.5604436
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0301013619753
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0903040859259

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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References:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Thrombomodulin_up

Assay Title: Bioseek human umbilical vein endothelium thrombomodulin assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Thrombomodulin was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Thrombomodulin_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene THBD. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Thrombomodulin, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.26162362
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0301013619753
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0903040859259

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_TissueFactor_down

Assay Title: Bioseek human umbilical vein endothelium coagulation factor III (thromboplastin, tissue factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_TissueFactor was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_TissueFactor_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene F3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "coagulation factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_TissueFactor, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.15552705
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04584406764
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.13753220292

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_TissueFactor_up

Assay Title: Bioseek human umbilical vein endothelium coagulation factor III (thromboplastin, tissue factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_TissueFactor was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_TissueFactor_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene F3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "coagulation factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_TissueFactor, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.59448808
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_uPAR_down

Assay Title: Bioseek human umbilical vein endothelium plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_uPAR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_uPAR, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.055609265
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04103210053089
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12309630159267

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_uPAR_up

Assay Title: Bioseek human umbilical vein endothelium plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_uPAR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_uPAR, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.43478277
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_VCAM1_down

Assay Title: Bioseek human umbilical vein endothelium vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_VCAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_VCAM1, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.5857159
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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1. Assay Descriptions

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Assay Throughput:

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Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
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Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.20014524
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.01897680541974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Vis_down

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Vis was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Vis_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, loss-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Vis, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with optical microscopy: fluorescence microscopy signals by light microscopy technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_3C_Vis_up

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_3C_Vis was analyzed into 2 assay endpoints. This assay endpoint, BSK_3C_Vis_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of morphology reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_3C_Vis, is one of 13 assay component(s) measured or calculated from the BSK_3C assay. It is designed to make measurements of cell phenotype, a form of morphology reporter, as detected with optical microscopy: fluorescence microscopy signals by light microscopy technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.17609125
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_Eotaxin3_down

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-C motif) ligand 26 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_Eotaxin3 was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_Eotaxin3_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL26. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_Eotaxin3, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3410609
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0275372927487
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0826118782461

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_Eotaxin3_up

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-C motif) ligand 26 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_Eotaxin3 was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_Eotaxin3_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL26. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_Eotaxin3, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.1319062
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0275372927487
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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_MCP1_down

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_MCP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_MCP1, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.889
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0567665582694
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1702996748082

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_MCP1_up

Assay Title: Bioseek human umbilical vein endothelium chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_MCP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_MCP1, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.51119304
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_Pselectin_down

Assay Title: Bioseek human umbilical vein endothelium selectin P (granule membrane protein 140kDa, antigen CD62) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_Pselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_Pselectin_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELP. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_Pselectin, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.88708392
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04150105602888
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12450316808664

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_Pselectin_up

Assay Title: Bioseek human umbilical vein endothelium selectin P (granule membrane protein 140kDa, antigen CD62) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_Pselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_Pselectin_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELP. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_Pselectin, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.44053206
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04150105602888
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12450316808664

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_SRB_down

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_SRB, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.39077475
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0229418168931
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_SRB_up

Assay Title: Bioseek human umbilical vein endothelium unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_SRB, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.116
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0229418168931
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_uPAR_down

Assay Title: Bioseek human umbilical vein endothelium plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_uPAR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_uPAR, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.01945125
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0375520200153
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1126560600459

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_uPAR_up

Assay Title: Bioseek human umbilical vein endothelium plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_uPAR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_uPAR, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.40480685
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1126560600459

Chemical Library Scope and Limitations:

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availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_VCAM1_down

Assay Title: Bioseek human umbilical vein endothelium vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_VCAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_VCAM1, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.63082125
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.023029781775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_VCAM1_up

Assay Title: Bioseek human umbilical vein endothelium vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_VCAM1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_VCAM1, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.08557246
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.023029781775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_VEGFRII_down

Assay Title: Bioseek human umbilical vein endothelium kinase insert domain receptor (a type III receptor tyrosine kinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_VEGFRII was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_VEGFRII_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene KDR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_VEGFRII, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.41383207
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0348840783501
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1046522350503

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_4H_VEGFRII_up

Assay Title: Bioseek human umbilical vein endothelium kinase insert domain receptor (a type III receptor tyrosine kinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_4H_VEGFRII was analyzed into 2 assay endpoints. This assay endpoint, BSK_4H_VEGFRII_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene KDR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium primary cell

Basic Procedure:
BSK_4H_VEGFRII, is one of 7 assay component(s) measured or calculated from the BSK_4H assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_4H is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium, a human vascular primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.41494396
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0348840783501
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1046522350503

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_HLADR_down

Assay Title: Bioseek human bronchial epithelial cells major histocompatibility complex, class II, DR alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_HLADR was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_HLADR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene HLA-DRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "MHC Class II".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_HLADR, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.2224989
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0264369307503
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0793107922509

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_HLADR_up

Assay Title: Bioseek human bronchial epithelial cells major histocompatibility complex, class II, DR alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_HLADR was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_HLADR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene HLA-DRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "MHC Class II".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_HLADR, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.13980329
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0264369307503
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0793107922509

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_IL1a_down

Assay Title: Bioseek human bronchial epithelial cells interleukin 1, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_IL1a was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_IL1a_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_IL1a, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0935905
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

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Nominal number of tested concentrations:	4
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0271961954145
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Chemical Library Scope and Limitations:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_IL1a_up

Assay Title: Bioseek human bronchial epithelial cells interleukin 1, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_IL1a was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_IL1a_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_IL1a, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.12
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0271961954145
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0815885862435

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_IP10_down

Assay Title: Bioseek human bronchial epithelial cells chemokine (C-X-C motif) ligand 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_IP10 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_IP10_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_IP10, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.7384221
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0297148689117
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0891446067351

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_IP10_up

Assay Title: Bioseek human bronchial epithelial cells chemokine (C-X-C motif) ligand 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_IP10 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_IP10_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_IP10, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.099
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_MIG_down

Assay Title: Bioseek human bronchial epithelial cells chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_MIG_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_MIG, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.38850585
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00717907952328
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_MIG_up

Assay Title: Bioseek human bronchial epithelial cells chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_MIG_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

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Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_MIG, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

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2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.043044386
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_MMP1_down

Assay Title: Bioseek human bronchial epithelial cells matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_MMP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_MMP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene MMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_MMP1, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.85596115
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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adherent bronchial epithelial cells primary cell

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2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.31558797
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02977671623787
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.08933014871361

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_PAI1_down

Assay Title: Bioseek human bronchial epithelial cells serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_PAI1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_PAI1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SERPINE1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_PAI1, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.83260345
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0384368386605
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1153105159815

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_PAI1_up

Assay Title: Bioseek human bronchial epithelial cells serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_PAI1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_PAI1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SERPINE1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_PAI1, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.23570101
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0384368386605
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1153105159815

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_SRB_down

Assay Title: Bioseek human bronchial epithelial cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_SRB, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3637528
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.005504480273208
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_SRB_up

Assay Title: Bioseek human bronchial epithelial cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_SRB, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.0509007145
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_TGFb1_down

Assay Title: Bioseek human bronchial epithelial cells transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_TGFb1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_TGFb1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene TGFB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "growth factor" intended target family, where the subfamily is "transforming growth factor beta".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_TGFb1, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.804
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.034880351835
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.104641055505

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_TGFb1_up

Assay Title: Bioseek human bronchial epithelial cells transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_TGFb1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_TGFb1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene TGFB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "growth factor" intended target family, where the subfamily is "transforming growth factor beta".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_TGFb1, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.171
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_tPA_down

Assay Title: Bioseek human bronchial epithelial cells plasminogen activator, tissue assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_tPA was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_tPA_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAT. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_tPA, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.72405374
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0464028210324
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1392084630972

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_tPA_up

Assay Title: Bioseek human bronchial epithelial cells plasminogen activator, tissue assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_tPA was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_tPA_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAT. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_tPA, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.32036966
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0464028210324
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1392084630972

Chemical Library Scope and Limitations:

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_uPA_down

Assay Title: Bioseek human bronchial epithelial cells plasminogen activator, urokinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_uPA was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_uPA_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAU. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_uPA, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.852257065
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.08859059477163

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_uPA_up

Assay Title: Bioseek human bronchial epithelial cells plasminogen activator, urokinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_uPA was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_uPA_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAU. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_uPA, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.184951565
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02953019825721
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.08859059477163

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_uPAR_down

Assay Title: Bioseek human bronchial epithelial cells plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_uPAR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_uPAR, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.96321863
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.040486699953
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.121460099859

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.2 Abbreviations and Definitions

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_BE3C_uPAR_up

Assay Title: Bioseek human bronchial epithelial cells plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_BE3C_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_BE3C_uPAR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent bronchial epithelial cells primary cell

Basic Procedure:
BSK_BE3C_uPAR, is one of 11 assay component(s) measured or calculated from the BSK_BE3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_BE3C is a cell-based, multiplexed-readout assay that uses bronchial epithelial cell, a human lung primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.2775738
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_HLADR_down

Assay Title: Bioseek human coronary artery smooth muscle cells major histocompatibility complex, class II, DR alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_HLADR was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_HLADR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene HLA-DRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "MHC Class II".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_HLADR, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.4288599
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03669291328647
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.11007873985941

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_HLADR_up

Assay Title: Bioseek human coronary artery smooth muscle cells major histocompatibility complex, class II, DR alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_HLADR was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_HLADR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene HLA-DRA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "MHC Class II".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_HLADR, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.211588085
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_IL6_down

Assay Title: Bioseek human coronary artery smooth muscle cells interleukin 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_IL6 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_IL6_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_IL6, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0793362
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04083462643932
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12250387931796

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_IL6_up

Assay Title: Bioseek human coronary artery smooth muscle cells interleukin 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_IL6 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_IL6_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_IL6, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.451
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04083462643932
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12250387931796

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_IL8_down

Assay Title: Bioseek human coronary artery smooth muscle cells chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_IL8_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_IL8, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.864
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.01644225898455
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_IL8_up

Assay Title: Bioseek human coronary artery smooth muscle cells chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_IL8_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_IL8, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.137
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Chemical Library Scope and Limitations:

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_LDLR_down

Assay Title: Bioseek human coronary artery smooth muscle cells low density lipoprotein receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_LDLR was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_LDLR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene LDLR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "misc protein" intended target family, where the subfamily is "LDL receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_LDLR, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.51656345
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_LDLR_up

Assay Title: Bioseek human coronary artery smooth muscle cells low density lipoprotein receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_LDLR was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_LDLR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene LDLR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "misc protein" intended target family, where the subfamily is "LDL receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_LDLR, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.27676
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0248248219338
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_MCP1_down

Assay Title: Bioseek human coronary artery smooth muscle cells chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_MCP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_MCP1, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3325744
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03149030798655
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.09447092395965

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_MCP1_up

Assay Title: Bioseek human coronary artery smooth muscle cells chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_MCP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_MCP1, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.33279586
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_MCSF_down

Assay Title: Bioseek human coronary artery smooth muscle cells colony stimulating factor 1 (macrophage) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_MCSF was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_MCSF_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "colony stimulating factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_MCSF, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0328479
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0322492587102
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0967477761306

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_MCSF_up

Assay Title: Bioseek human coronary artery smooth muscle cells colony stimulating factor 1 (macrophage) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_MCSF was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_MCSF_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "colony stimulating factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_MCSF, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.27056522
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0322492587102
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0967477761306

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_MIG_down

Assay Title: Bioseek human coronary artery smooth muscle cells chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_MIG_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_MIG, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.9172763
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

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U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_MIG_up

Assay Title: Bioseek human coronary artery smooth muscle cells chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_MIG_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_MIG, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.07649083
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.015154425552
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_Proliferation_down

Assay Title: Bioseek human coronary artery smooth muscle cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_Proliferation_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_Proliferation, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.512
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03259366409565
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.09778099228695

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_Proliferation_up

Assay Title: Bioseek human coronary artery smooth muscle cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_Proliferation_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_Proliferation, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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None reported.

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Assay Objectives:

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Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.292
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_SAA_down

Assay Title: Bioseek human coronary artery smooth muscle cells serum amyloid A1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_SAA was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_SAA_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SAA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "apolipoproteins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_SAA, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.800077825
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03348698471055
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.10046095413165

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_SAA_up

Assay Title: Bioseek human coronary artery smooth muscle cells serum amyloid A1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_SAA was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_SAA_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SAA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "apolipoproteins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_SAA, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.756181565
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03348698471055
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.10046095413165

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_SRB_down

Assay Title: Bioseek human coronary artery smooth muscle cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_SRB, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.451599
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.01670137528458
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_SRB_up

Assay Title: Bioseek human coronary artery smooth muscle cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_SRB, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.17115746
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.01670137528458
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_Thrombomodulin_down

Assay Title: Bioseek human coronary artery smooth muscle cells thrombomodulin assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_Thrombomodulin was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_Thrombomodulin_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene THBD. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_Thrombomodulin, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.57925487
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0337185316188
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1011555948564

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_Thrombomodulin_up

Assay Title: Bioseek human coronary artery smooth muscle cells thrombomodulin assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_Thrombomodulin was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_Thrombomodulin_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene THBD. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_Thrombomodulin, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.629
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_TissueFactor_down

Assay Title: Bioseek human coronary artery smooth muscle cells coagulation factor III (thromboplastin, tissue factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_TissueFactor was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_TissueFactor_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene F3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "coagulation factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_TissueFactor, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.800554335
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
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Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04000840578
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12002521734

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_TissueFactor_up

Assay Title: Bioseek human coronary artery smooth muscle cells coagulation factor III (thromboplastin, tissue factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_TissueFactor was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_TissueFactor_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene F3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "coagulation factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_TissueFactor, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.31915455
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04000840578
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12002521734

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_uPAR_down

Assay Title: Bioseek human coronary artery smooth muscle cells plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_uPAR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_uPAR, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.337188
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0377505505335
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1132516516005

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_uPAR_up

Assay Title: Bioseek human coronary artery smooth muscle cells plasminogen activator, urokinase receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_uPAR was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_uPAR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAUR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_uPAR, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.28730634
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_VCAM1_down

Assay Title: Bioseek human coronary artery smooth muscle cells vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_VCAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_VCAM1, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.35207875
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.027979001068368
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.083937003205104

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_CASM3C_VCAM1_up

Assay Title: Bioseek human coronary artery smooth muscle cells vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_CASM3C_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_CASM3C_VCAM1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent coronary artery smooth muscle cells primary cell

Basic Procedure:
BSK_CASM3C_VCAM1, is one of 14 assay component(s) measured or calculated from the BSK_CASM3C assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_CASM3C is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and coronary artery smooth muscle cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.21622041
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.027979001068368
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.083937003205104

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_CollagenIII_down

Assay Title: Bioseek human foreskin fibroblast collagen, type III, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_CollagenIII was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_CollagenIII_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene COL3A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "collagen".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_CollagenIII, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.1332279
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0480236571738
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1440709715214

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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UGT, UDP-glucuronosyltransferase

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_CollagenIII_up

Assay Title: Bioseek human foreskin fibroblast collagen, type III, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_CollagenIII was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_CollagenIII_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene COL3A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "collagen".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_CollagenIII, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.28650303
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0480236571738
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1440709715214

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_EGFR_down

Assay Title: Bioseek human foreskin fibroblast epidermal growth factor receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_EGFR was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_EGFR_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene EGFR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_EGFR, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.497715225
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04256010864
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12768032592

Chemical Library Scope and Limitations:

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availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_EGFR_up

Assay Title: Bioseek human foreskin fibroblast epidermal growth factor receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_EGFR was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_EGFR_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene EGFR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_EGFR, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.41
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_IL8_down

Assay Title: Bioseek human foreskin fibroblast chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_IL8_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_IL8, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.54420495
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.008269695880383
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_IL8_up

Assay Title: Bioseek human foreskin fibroblast chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_IL8_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_IL8, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.064
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.008269695880383
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_IP10_down

Assay Title: Bioseek human foreskin fibroblast chemokine (C-X-C motif) ligand 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_IP10 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_IP10_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_IP10, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.78274145
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02832826466715
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.08498479400145

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_IP10_up

Assay Title: Bioseek human foreskin fibroblast chemokine (C-X-C motif) ligand 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_IP10 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_IP10_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_IP10, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.190988585
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02832826466715
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.08498479400145

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_MCSF_down

Assay Title: Bioseek human foreskin fibroblast colony stimulating factor 1 (macrophage) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_MCSF was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_MCSF_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "colony stimulating factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_MCSF, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.2501971
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0372181014303
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1116543042909

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_MCSF_up

Assay Title: Bioseek human foreskin fibroblast colony stimulating factor 1 (macrophage) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_MCSF was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_MCSF_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "colony stimulating factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_MCSF, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.144
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Chemical Library Scope and Limitations:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_MIG_down

Assay Title: Bioseek human foreskin fibroblast chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_MIG_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_MIG, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.74010585
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_MIG_up

Assay Title: Bioseek human foreskin fibroblast chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_MIG_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_MIG, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.12
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.01472645467776
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_MMP1_down

Assay Title: Bioseek human foreskin fibroblast matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_MMP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_MMP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene MMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_MMP1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.94125273
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0282963394707
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0848890184121

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_MMP1_up

Assay Title: Bioseek human foreskin fibroblast matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_MMP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_MMP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene MMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_MMP1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.22363375
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_PAI1_down

Assay Title: Bioseek human foreskin fibroblast serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_PAI1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_PAI1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SERPINE1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_PAI1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.34963875
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0569381158458
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1708143475374

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_PAI1_up

Assay Title: Bioseek human foreskin fibroblast serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_PAI1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_PAI1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SERPINE1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "plasmogen activator inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_PAI1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.375
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0569381158458
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1708143475374

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_Proliferation_down

Assay Title: Bioseek human foreskin fibroblast unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_Proliferation_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_Proliferation, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.6240079
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04442042878875
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.13326128636625

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25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_Proliferation_up

Assay Title: Bioseek human foreskin fibroblast unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_Proliferation_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_Proliferation, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.323750875
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04442042878875
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.13326128636625

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_SRB_down

Assay Title: Bioseek human foreskin fibroblast unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_SRB, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3678178
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02296976212398
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_SRB_up

Assay Title: Bioseek human foreskin fibroblast unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_SRB, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.15050885
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_TIMP1_down

Assay Title: Bioseek human foreskin fibroblast TIMP metallopeptidase inhibitor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_TIMP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_TIMP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene TIMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease inhibitor" intended target family, where the subfamily is "metalloproteinase inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_TIMP1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.99043017
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0451776003924
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1355328011772

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_TIMP1_up

Assay Title: Bioseek human foreskin fibroblast TIMP metallopeptidase inhibitor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_TIMP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_TIMP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene TIMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease inhibitor" intended target family, where the subfamily is "metalloproteinase inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_TIMP1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.2940745
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0451776003924
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1355328011772

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_VCAM1_down

Assay Title: Bioseek human foreskin fibroblast vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_VCAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_VCAM1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.2998879
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
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Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.048068234508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.144204703524

Chemical Library Scope and Limitations:

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_hDFCGF_VCAM1_up

Assay Title: Bioseek human foreskin fibroblast vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_hDFCGF_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_hDFCGF_VCAM1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent foreskin fibroblast primary cell

Basic Procedure:
BSK_hDFCGF_VCAM1, is one of 12 assay component(s) measured or calculated from the BSK_hDFCGF assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_hDFCGF is a cell-based, multiplexed-readout assay that uses foreskin fibroblast, a human skin primary cell, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.21893832
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.048068234508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.144204703524

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_ICAM1_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts intercellular adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_ICAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_ICAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene ICAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_ICAM1, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.6354748
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02110495177287
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_ICAM1_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts intercellular adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_ICAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_ICAM1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene ICAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_ICAM1, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.13107166
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02110495177287
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_IL1a_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts interleukin 1, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_IL1a was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_IL1a_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_IL1a, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.2097417
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_IL1a_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts interleukin 1, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_IL1a was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_IL1a_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_IL1a, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.14547459
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0296319641436
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0888958924308

Chemical Library Scope and Limitations:

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_IP10_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts chemokine (C-X-C motif) ligand 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_IP10 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_IP10_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_IP10, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.68192735
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0140925155259
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_IP10_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts chemokine (C-X-C motif) ligand 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_IP10 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_IP10_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_IP10, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.178
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_MCP1_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_MCP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_MCP1, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.30687205
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0402316386381
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1206949159143

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_MCP1_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_MCP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_MCP1, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.386
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0402316386381
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1206949159143

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_MMP9_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_MMP9 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_MMP9_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene MMP9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_MMP9, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.57115545
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0299145410319
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0897436230957

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

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4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_MMP9_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_MMP9 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_MMP9_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene MMP9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_MMP9, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.113
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0299145410319
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0897436230957

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_SRB_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_SRB, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0087901
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.004455579113244
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_SRB_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_SRB, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.082
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_TGFb1_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_TGFb1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_TGFb1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene TGFB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "growth factor" intended target family, where the subfamily is "transforming growth factor beta".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_TGFb1, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.9206665
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0353563220325
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1060689660975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_TGFb1_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_TGFb1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_TGFb1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene TGFB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "growth factor" intended target family, where the subfamily is "transforming growth factor beta".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_TGFb1, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.6600929
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0353563220325
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1060689660975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_TIMP2_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts TIMP metallopeptidase inhibitor 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_TIMP2 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_TIMP2_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene TIMP2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease inhibitor" intended target family, where the subfamily is "metalloproteinase inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_TIMP2, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.1045663
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0320903714334
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0962711143002

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_TIMP2_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts TIMP metallopeptidase inhibitor 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_TIMP2 was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_TIMP2_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene TIMP2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease inhibitor" intended target family, where the subfamily is "metalloproteinase inhibitor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_TIMP2, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.159
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0320903714334
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0962711143002

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_uPA_down

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts plasminogen activator, urokinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_uPA was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_uPA_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAU. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_uPA, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.4587075
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.021315629573868
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_KF3CT_uPA_up

Assay Title: Bioseek human keratinocytes and foreskin fibroblasts plasminogen activator, urokinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_KF3CT_uPA was analyzed into 2 assay endpoints. This assay endpoint, BSK_KF3CT_uPA_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PLAU. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent keratinocytes and foreskin fibroblasts primary cell co-culture

Basic Procedure:
BSK_KF3CT_uPA, is one of 9 assay component(s) measured or calculated from the BSK_KF3CT assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_KF3CT is a cell-based, multiplexed-readout assay that uses keratinocytes and foreskin fibroblasts, a human skin primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.1113362
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	4
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Standard minimum concentration tested:	1 µM
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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_CD40_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD40 molecule, TNF receptor superfamily member 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_CD40 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_CD40_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD40. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_CD40, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.949602815
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_CD40_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD40 molecule, TNF receptor superfamily member 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_CD40 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_CD40_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD40. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_CD40, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.31714723
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0476603023071
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1429809069213

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_Eselectin_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells selectin E assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_Eselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_Eselectin_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_Eselectin, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.5463713
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0252446431041
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_Eselectin_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells selectin E assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_Eselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_Eselectin_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_Eselectin, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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None reported.

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Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.11940922
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_IL1a_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells interleukin 1, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_IL1a was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_IL1a_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_IL1a, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0789886
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0413992543377
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1241977630131

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_IL1a_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells interleukin 1, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_IL1a was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_IL1a_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene IL1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_IL1a, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.487044
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0413992543377
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1241977630131

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_IL8_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_IL8_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_IL8, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.38222185
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.029411899208811
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.088235697626433

Chemical Library Scope and Limitations:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_IL8_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_IL8_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_IL8, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.218214515
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.029411899208811
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.088235697626433

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_MCP1_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_MCP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

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Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_MCP1, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.406
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0314694644838
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0944083934514

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BSK_LPS_MCP1_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

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Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
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Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.23404112
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

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Biological Response:

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BSK_LPS_MCSF_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells colony stimulating factor 1 (macrophage) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

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96-well plate

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adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

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Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.98669925
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.036823070614449
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.110469211843347

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_MCSF_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells colony stimulating factor 1 (macrophage) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_MCSF was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_MCSF_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "colony stimulating factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_MCSF, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.26982114
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.036823070614449
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.110469211843347

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_PGE2_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells prostaglandin E receptor 2 (subtype EP2), 53kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_PGE2 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_PGE2_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene PTGER2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_PGE2, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0359748
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.048135233202
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.144405699606

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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EPA NCCT

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_PGE2_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells prostaglandin E receptor 2 (subtype EP2), 53kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_PGE2 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_PGE2_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene PTGER2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_PGE2, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.484
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.048135233202
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.144405699606

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_SRB_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_SRB, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3749337
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

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Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0208862171973
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_SRB_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_SRB, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.113
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	4
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_TissueFactor_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells coagulation factor III (thromboplastin, tissue factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_TissueFactor was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_TissueFactor_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene F3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "coagulation factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_TissueFactor, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.12776655
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_TissueFactor_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells coagulation factor III (thromboplastin, tissue factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_TissueFactor was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_TissueFactor_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene F3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "coagulation factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_TissueFactor, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.44227785
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0408420065256
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1225260195768

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_TNFa_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells tumor necrosis factor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_TNFa was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_TNFa_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene TNF. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_TNFa, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.0741055
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.05057530569651
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.15172591708953

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

25 September 2018

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_TNFa_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells tumor necrosis factor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_TNFa was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_TNFa_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene TNF. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_TNFa, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.4421049
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_VCAM1_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_VCAM1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_VCAM1, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.58591015
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02313823589238
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_LPS_VCAM1_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells vascular cell adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_LPS_VCAM1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_LPS_VCAM1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene VCAM1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "Immunoglobulin CAM".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_LPS_VCAM1, is one of 11 assay component(s) measured or calculated from the BSK_LPS assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_LPS is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.1166973775
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02313823589238
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_CD38_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD38 molecule assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_CD38 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_CD38_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD38. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "other cytokine".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_CD38, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.2047119
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02475238550778
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_CD38_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD38 molecule assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_CD38 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_CD38_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD38. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "other cytokine".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_CD38, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.3716924
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.02475238550778
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_CD40_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD40 molecule, TNF receptor superfamily member 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_CD40 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_CD40_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD40. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_CD40, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.1938512
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03534421104375
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.10603263313125

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_CD40_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD40 molecule, TNF receptor superfamily member 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_CD40 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_CD40_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD40. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_CD40, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.24001062
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.03534421104375
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.10603263313125

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_CD69_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD69 molecule assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_CD69 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_CD69_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD69. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_CD69, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.18972525
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0393111508608
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1179334525824

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_CD69_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells CD69 molecule assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_CD69 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_CD69_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CD69. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "inflammatory factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_CD69, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.212
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	4
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Standard minimum concentration tested:	1 µM
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4.2 Abbreviations and Definitions

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_Eselectin_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells selectin E assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_Eselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_Eselectin_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_Eselectin, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.41268635
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_Eselectin_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells selectin E assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_Eselectin was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_Eselectin_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene SELE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell adhesion molecules" intended target family, where the subfamily is "selectins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_Eselectin, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

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Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.274837875
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.04260230677185
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.12780692031555

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_IL8_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_IL8_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_IL8, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.008
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0514308921399
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1542926764197

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_IL8_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-X-C motif) ligand 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_IL8 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_IL8_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "interleukins".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_IL8, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.370428135
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1542926764197

Chemical Library Scope and Limitations:

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_MCP1_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_MCP1_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_MCP1, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.16421415
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_MCP1_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-C motif) ligand 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_MCP1 was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_MCP1_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CCL2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_MCP1, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.2768968
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0340299280272
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1020897840816

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_MIG_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_MIG_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_MIG, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.68065635
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.00863239780263
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_MIG_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells chemokine (C-X-C motif) ligand 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_MIG was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_MIG_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, gain-of-signal activity can be used to understand changes in the signaling as they relate to the gene CXCL9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cytokine" intended target family, where the subfamily is "chemotactic factor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_MIG, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of enzyme-linked immunosorbent assay, a form of binding reporter, as detected with fluorescence intensity signals by Enzyme-linked immunosorbent assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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None reported.

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Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.072
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_PBMCCytotoxicity_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_PBMCCytotoxicity was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_PBMCCytotoxicity_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, loss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_PBMCCytotoxicity, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of dehydrogenase activity determination, a form of viability reporter, as detected with fluorescence intensity signals by Alamar Blue Reduction technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.1296256
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0204508168662
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_PBMCCytotoxicity_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_PBMCCytotoxicity was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_PBMCCytotoxicity_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the viability. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_PBMCCytotoxicity, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of dehydrogenase activity determination, a form of viability reporter, as detected with fluorescence intensity signals by Alamar Blue Reduction technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.33812026
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0204508168662
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_Proliferation_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_Proliferation_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_Proliferation, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.43300975
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0401013381132
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1203040143396

Chemical Library Scope and Limitations:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_Proliferation_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_Proliferation was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_Proliferation_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for gain-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "proliferation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_Proliferation, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.18463312
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0401013381132
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.1203040143396

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

References:

Houck KA, Dix DJ, Judson RS, Kavlock RJ, Yang J, Berg EL. Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. J Biomol Screen. 2009 Oct;14(9):1054-66. doi: 10.1177/1087057109345525. Epub 2009 Sep 22. PubMed PMID: 19773588.|Kleinstreuer NC, Yang J, Berg EL, Knudsen TB, Richard AM, Martin MT, Reif DM, Judson RS, Polokoff M, Dix DJ, Kavlock RJ, Houck KA. Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms. Nat Biotechnol. 2014 Jun;32(6):583-91. doi: 10.1038/nbt.2914. Epub 2014 May 18. PubMed PMID: 24837663.|Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Melrose J, Nguyen D, Ota KS, Plavec I, Wang Y, Watson SR, Butcher EC, Berg EL. An integrative biology approach for analysis of drug action in models of human vascular inflammation. FASEB J. 2004 Aug;18(11):1279-81. Epub 2004 Jun 18. PubMed PMID: 15208272.|Kunkel EJ, Plavec I, Nguyen D, Melrose J, Rosler ES, Kao LT, Wang Y, Hytopoulos E, Bishop AC, Bateman R, Shokat KM, Butcher EC, Berg EL. Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. Assay Drug Dev Technol. 2004 Aug;2(4):431-41. PubMed PMID: 15357924.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_SRB_down

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_SRB_down, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of total protein for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell cycle" intended target family, where the subfamily is "cytotoxicity".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_SRB, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.3578558
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	1 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0185276867798715
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

BSK_SAg_SRB_up

Assay Title: Bioseek human umbilical vein endothelium and peripheral blood mononuclear cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component BSK_SAg_SRB was analyzed into 2 assay endpoints. This assay endpoint, BSK_SAg_SRB_up, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cell morphology" intended target family, where the subfamily is "cell conformation".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent umbilical vein endothelium and peripheral blood mononuclear cells primary cell co-culture

Basic Procedure:
BSK_SAg_SRB, is one of 10 assay component(s) measured or calculated from the BSK_SAg assay. It is designed to make measurements of protein content, a form of viability reporter, as detected with absorbance signals by Sulforhodamine staining technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Bioseek is a division of DiscoveRx Corporation and developed the BioMAPÃ,Â® system providing uniquely informative biological activity profiles.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

BSK_SAg is a cell-based, multiplexed-readout assay that uses umbilical vein endothelium and peripheral blood mononuclear cells, a human vascular primary cell co-culture, with measurements taken at 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.142
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.0791812460476248

Chemical Library Scope and Limitations:

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availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_11DCORT_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	375.998
Neutral control median absolute deviation, by plate:	8.19878
Positive control well median response value, by plate:	862.42
Positive control well median absolute deviation, by plate:	28.3065
Negative control well median, by plate:	5.45445228669727
Negative control well median absolute deviation value, by plate:	1.20461
Z' (median across all plates, using positive control wells):	0.79
Z' (median across all plates, using negative control wells):	0.91
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	-35
Signal-to-noise (median across all plates, using positive control wells):	58.64
Signal-to-noise (median across all plates, using negative control wells):	-42.84
Signal-to-background (median across all plates, using positive control wells):	2.32
Signal-to-background (median across all plates, using negative control wells):	0.07
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0675346229668387
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.405207737801032

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_11DCORT_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	375.998
Neutral control median absolute deviation, by plate:	8.19878
Positive control well median response value, by plate:	862.42
Positive control well median absolute deviation, by plate:	28.3065
Negative control well median, by plate:	0.540081814433397
Negative control well median absolute deviation value, by plate:	1.20461
Z' (median across all plates, using positive control wells):	0.79
Z' (median across all plates, using negative control wells):	0.91
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	-35
Signal-to-noise (median across all plates, using positive control wells):	58.64
Signal-to-noise (median across all plates, using negative control wells):	-42.84
Signal-to-background (median across all plates, using positive control wells):	2.32
Signal-to-background (median across all plates, using negative control wells):	0.07
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0675346229668387
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.405207737801032

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_ANDR_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	99.025
Neutral control median absolute deviation, by plate:	4.08827
Positive control well median response value, by plate:	268.722
Positive control well median absolute deviation, by plate:	8.50642
Negative control well median, by plate:	5.2418783101078
Negative control well median absolute deviation value, by plate:	0.096369
Z' (median across all plates, using positive control wells):	0.77
Z' (median across all plates, using negative control wells):	0.86
SSMD (median across all plates, using positive control wells):	18
SSMD (median across all plates, using negative control wells):	-22
Signal-to-noise (median across all plates, using positive control wells):	39.67
Signal-to-noise (median across all plates, using negative control wells):	-22.23
Signal-to-background (median across all plates, using positive control wells):	2.71
Signal-to-background (median across all plates, using negative control wells):	0.02
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0932518549539463
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.559511129723678

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_ANDR_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	99.025
Neutral control median absolute deviation, by plate:	4.08827
Positive control well median response value, by plate:	268.722
Positive control well median absolute deviation, by plate:	8.50642
Negative control well median, by plate:	0.520276595553919
Negative control well median absolute deviation value, by plate:	0.096369
Z' (median across all plates, using positive control wells):	0.77
Z' (median across all plates, using negative control wells):	0.86
SSMD (median across all plates, using positive control wells):	18
SSMD (median across all plates, using negative control wells):	-22
Signal-to-noise (median across all plates, using positive control wells):	39.67
Signal-to-noise (median across all plates, using negative control wells):	-22.23
Signal-to-background (median across all plates, using positive control wells):	2.71
Signal-to-background (median across all plates, using negative control wells):	0.02
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.0932518549539463
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.559511129723678

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_CORTIC_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.58
Neutral control median absolute deviation, by plate:	0.126021
Positive control well median response value, by plate:	13.67
Positive control well median absolute deviation, by plate:	0.507791
Negative control well median, by plate:	2.12847455794881
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.8
Z' (median across all plates, using negative control wells):	0.9
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	-32
Signal-to-noise (median across all plates, using positive control wells):	90.5
Signal-to-noise (median across all plates, using negative control wells):	-32.42
Signal-to-background (median across all plates, using positive control wells):	5.27
Signal-to-background (median across all plates, using negative control wells):	0.19
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.163204816089315
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.97922889653589

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_CORTIC_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.58
Neutral control median absolute deviation, by plate:	0.126021
Positive control well median response value, by plate:	13.67
Positive control well median absolute deviation, by plate:	0.507791
Negative control well median, by plate:	1.76383379961801
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.8
Z' (median across all plates, using negative control wells):	0.9
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	-32
Signal-to-noise (median across all plates, using positive control wells):	90.5
Signal-to-noise (median across all plates, using negative control wells):	-32.42
Signal-to-background (median across all plates, using positive control wells):	5.27
Signal-to-background (median across all plates, using negative control wells):	0.19
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.163204816089315
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.97922889653589

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_CORTISOL_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.1975
Neutral control median absolute deviation, by plate:	1.54561
Positive control well median response value, by plate:	101.325
Positive control well median absolute deviation, by plate:	2.82806
Negative control well median, by plate:	5.43937856579735
Negative control well median absolute deviation value, by plate:	0.0778365
Z' (median across all plates, using positive control wells):	0.78
Z' (median across all plates, using negative control wells):	0.83
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	-18
Signal-to-noise (median across all plates, using positive control wells):	46.76
Signal-to-noise (median across all plates, using negative control wells):	-17.99
Signal-to-background (median across all plates, using positive control wells):	3.56
Signal-to-background (median across all plates, using negative control wells):	0.03
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.112302593387112
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.673815560322672

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_CORTISOL_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.1975
Neutral control median absolute deviation, by plate:	1.54561
Positive control well median response value, by plate:	101.325
Positive control well median absolute deviation, by plate:	2.82806
Negative control well median, by plate:	0.664887134573989
Negative control well median absolute deviation value, by plate:	0.0778365
Z' (median across all plates, using positive control wells):	0.78
Z' (median across all plates, using negative control wells):	0.83
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	-18
Signal-to-noise (median across all plates, using positive control wells):	46.76
Signal-to-noise (median across all plates, using negative control wells):	-17.99
Signal-to-background (median across all plates, using positive control wells):	3.56
Signal-to-background (median across all plates, using negative control wells):	0.03
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.112302593387112
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.673815560322672

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_DHEA_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.925
Neutral control median absolute deviation, by plate:	0.437367
Positive control well median response value, by plate:	20.5825
Positive control well median absolute deviation, by plate:	1.44924
Negative control well median, by plate:	0.840411243119338
Negative control well median absolute deviation value, by plate:	1.33434
Z' (median across all plates, using positive control wells):	0.55
Z' (median across all plates, using negative control wells):	0
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	0
Signal-to-noise (median across all plates, using positive control wells):	33.45
Signal-to-noise (median across all plates, using negative control wells):	-0.38
Signal-to-background (median across all plates, using positive control wells):	4.57
Signal-to-background (median across all plates, using negative control wells):	0.94
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.171870364320441
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03122218592265

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_DHEA_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.925
Neutral control median absolute deviation, by plate:	0.437367
Positive control well median response value, by plate:	20.5825
Positive control well median absolute deviation, by plate:	1.44924
Negative control well median, by plate:	2.61817182935506
Negative control well median absolute deviation value, by plate:	1.33434
Z' (median across all plates, using positive control wells):	0.55
Z' (median across all plates, using negative control wells):	0
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	0
Signal-to-noise (median across all plates, using positive control wells):	33.45
Signal-to-noise (median across all plates, using negative control wells):	-0.38
Signal-to-background (median across all plates, using positive control wells):	4.57
Signal-to-background (median across all plates, using negative control wells):	0.94
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.171870364320441
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.03122218592265

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_DOC_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	14.955
Neutral control median absolute deviation, by plate:	0.418835
Positive control well median response value, by plate:	31.7125
Positive control well median absolute deviation, by plate:	1.07118
Negative control well median, by plate:	4.58674146280741
Negative control well median absolute deviation value, by plate:	1.10083
Z' (median across all plates, using positive control wells):	0.76
Z' (median across all plates, using negative control wells):	0.65
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	10
Signal-to-noise (median across all plates, using positive control wells):	40.3
Signal-to-noise (median across all plates, using negative control wells):	31.6
Signal-to-background (median across all plates, using positive control wells):	2.16
Signal-to-background (median across all plates, using negative control wells):	1.91
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.104894412215344
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.629366473292064

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_DOC_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	14.955
Neutral control median absolute deviation, by plate:	0.418835
Positive control well median response value, by plate:	31.7125
Positive control well median absolute deviation, by plate:	1.07118
Negative control well median, by plate:	1.68551233911078
Negative control well median absolute deviation value, by plate:	1.10083
Z' (median across all plates, using positive control wells):	0.76
Z' (median across all plates, using negative control wells):	0.65
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	10
Signal-to-noise (median across all plates, using positive control wells):	40.3
Signal-to-noise (median across all plates, using negative control wells):	31.6
Signal-to-background (median across all plates, using positive control wells):	2.16
Signal-to-background (median across all plates, using negative control wells):	1.91
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.104894412215344
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.629366473292064

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_ESTRADIOL_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.28
Neutral control median absolute deviation, by plate:	0.022239
Positive control well median response value, by plate:	1.9375
Positive control well median absolute deviation, by plate:	0.114901
Negative control well median, by plate:	2.88264304936184
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.75
Z' (median across all plates, using negative control wells):	0.68
SSMD (median across all plates, using positive control wells):	14
SSMD (median across all plates, using negative control wells):	-10
Signal-to-noise (median across all plates, using positive control wells):	79.7
Signal-to-noise (median across all plates, using negative control wells):	-9.67
Signal-to-background (median across all plates, using positive control wells):	7.56
Signal-to-background (median across all plates, using negative control wells):	0.17
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.176872591204837
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.06123554722902

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_ESTRADIOL_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.28
Neutral control median absolute deviation, by plate:	0.022239
Positive control well median response value, by plate:	1.9375
Positive control well median absolute deviation, by plate:	0.114901
Negative control well median, by plate:	2.51349836618901
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.75
Z' (median across all plates, using negative control wells):	0.68
SSMD (median across all plates, using positive control wells):	14
SSMD (median across all plates, using negative control wells):	-10
Signal-to-noise (median across all plates, using positive control wells):	79.7
Signal-to-noise (median across all plates, using negative control wells):	-9.67
Signal-to-background (median across all plates, using positive control wells):	7.56
Signal-to-background (median across all plates, using negative control wells):	0.17
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.176872591204837
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.06123554722902

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_ESTRONE_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.1075
Neutral control median absolute deviation, by plate:	0.137141
Positive control well median response value, by plate:	18.0825
Positive control well median absolute deviation, by plate:	0.81543
Negative control well median, by plate:	5.54293952600098
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.81
Z' (median across all plates, using negative control wells):	0.74
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	-12
Signal-to-noise (median across all plates, using positive control wells):	100.55
Signal-to-noise (median across all plates, using negative control wells):	-13.5
Signal-to-background (median across all plates, using positive control wells):	8.56
Signal-to-background (median across all plates, using negative control wells):	0.02
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.20467616401251
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.22805698407506

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_ESTRONE_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.1075
Neutral control median absolute deviation, by plate:	0.137141
Positive control well median response value, by plate:	18.0825
Positive control well median absolute deviation, by plate:	0.81543
Negative control well median, by plate:	1.8096297184495
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.81
Z' (median across all plates, using negative control wells):	0.74
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	-12
Signal-to-noise (median across all plates, using positive control wells):	100.55
Signal-to-noise (median across all plates, using negative control wells):	-13.5
Signal-to-background (median across all plates, using positive control wells):	8.56
Signal-to-background (median across all plates, using negative control wells):	0.02
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.20467616401251
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1.22805698407506

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_MTT_Cytotoxicity_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.465
Neutral control median absolute deviation, by plate:	0.0133434
Positive control well median response value, by plate:	0.011
Positive control well median absolute deviation, by plate:	0.0022239
Negative control well median, by plate:	98.935298935299
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.89
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.85
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.96608141861838
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_MTT_Cytotoxicity_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.89
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.85
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_OHPREG_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.6475
Neutral control median absolute deviation, by plate:	0.355824
Positive control well median response value, by plate:	41.55
Positive control well median absolute deviation, by plate:	1.06006
Negative control well median, by plate:	1.03604549849361
Negative control well median absolute deviation value, by plate:	0.437367
Z' (median across all plates, using positive control wells):	0.84
Z' (median across all plates, using negative control wells):	0.72
SSMD (median across all plates, using positive control wells):	22
SSMD (median across all plates, using negative control wells):	11
Signal-to-noise (median across all plates, using positive control wells):	89.33
Signal-to-noise (median across all plates, using negative control wells):	17.19
Signal-to-background (median across all plates, using positive control wells):	3.98
Signal-to-background (median across all plates, using negative control wells):	2.09
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.102936749127555
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.61762049476533

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

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CEETOX_H295R_OHPREG_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Signal-to-background (median across all plates, using positive control wells):	3.98
Signal-to-background (median across all plates, using negative control wells):	2.09
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
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4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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CEETOX_H295R_OHPROG_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	16.3525
Neutral control median absolute deviation, by plate:	0.644931
Positive control well median response value, by plate:	38.7025
Positive control well median absolute deviation, by plate:	1.60491
Negative control well median, by plate:	4.96794488227857
Negative control well median absolute deviation value, by plate:	0.189031
Z' (median across all plates, using positive control wells):	0.64
Z' (median across all plates, using negative control wells):	0.48
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	-7
Signal-to-noise (median across all plates, using positive control wells):	33.92
Signal-to-noise (median across all plates, using negative control wells):	-7.9
Signal-to-background (median across all plates, using positive control wells):	2.3
Signal-to-background (median across all plates, using negative control wells):	0.65
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.1129800215436
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.6778801292616

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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CEETOX_H295R_OHPROG_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

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CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

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Experimental System:

adherent H295R cell line

Basic Procedure:
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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.605
Neutral control median absolute deviation, by plate:	0.392889
Positive control well median response value, by plate:	16.33
Positive control well median absolute deviation, by plate:	0.433661
Negative control well median, by plate:	1.56643121782956
Negative control well median absolute deviation value, by plate:	0.770952
Z' (median across all plates, using positive control wells):	0.7
Z' (median across all plates, using negative control wells):	0.63
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	10
Signal-to-noise (median across all plates, using positive control wells):	25.09
Signal-to-noise (median across all plates, using negative control wells):	19.18
Signal-to-background (median across all plates, using positive control wells):	2.46
Signal-to-background (median across all plates, using negative control wells):	2.3
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.112061281899255
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.67236769139553

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_PREG_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	6.605
Neutral control median absolute deviation, by plate:	0.392889
Positive control well median response value, by plate:	16.33
Positive control well median absolute deviation, by plate:	0.433661
Negative control well median, by plate:	1.74420026494095
Negative control well median absolute deviation value, by plate:	0.770952
Z' (median across all plates, using positive control wells):	0.7
Z' (median across all plates, using negative control wells):	0.63
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	10
Signal-to-noise (median across all plates, using positive control wells):	25.09
Signal-to-noise (median across all plates, using negative control wells):	19.18
Signal-to-background (median across all plates, using positive control wells):	2.46
Signal-to-background (median across all plates, using negative control wells):	2.3
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
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Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.112061281899255
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.67236769139553

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_PROG_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.8025
Neutral control median absolute deviation, by plate:	0.029652
Positive control well median response value, by plate:	1.17
Positive control well median absolute deviation, by plate:	0.029652
Negative control well median, by plate:	1.75973690182486
Negative control well median absolute deviation value, by plate:	0.467019
Z' (median across all plates, using positive control wells):	0.4
Z' (median across all plates, using negative control wells):	0.88
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	28
Signal-to-noise (median across all plates, using positive control wells):	11.4
Signal-to-noise (median across all plates, using negative control wells):	433.19
Signal-to-background (median across all plates, using positive control wells):	1.41
Signal-to-background (median across all plates, using negative control wells):	17.4
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.124141459512256
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.744848757073536

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_PROG_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.8025
Neutral control median absolute deviation, by plate:	0.029652
Positive control well median response value, by plate:	1.17
Positive control well median absolute deviation, by plate:	0.029652
Negative control well median, by plate:	3.79950137827042
Negative control well median absolute deviation value, by plate:	0.467019
Z' (median across all plates, using positive control wells):	0.4
Z' (median across all plates, using negative control wells):	0.88
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	28
Signal-to-noise (median across all plates, using positive control wells):	11.4
Signal-to-noise (median across all plates, using negative control wells):	433.19
Signal-to-background (median across all plates, using positive control wells):	1.41
Signal-to-background (median across all plates, using negative control wells):	17.4
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.124141459512256
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.744848757073536

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_TESTO_noMTC_dn

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.6975
Neutral control median absolute deviation, by plate:	0.133434
Positive control well median response value, by plate:	6.4325
Positive control well median absolute deviation, by plate:	0.378063
Negative control well median, by plate:	4.56695640711952
Negative control well median absolute deviation value, by plate:	0.022239
Z' (median across all plates, using positive control wells):	0.52
Z' (median across all plates, using negative control wells):	0.81
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	-19
Signal-to-noise (median across all plates, using positive control wells):	28.04
Signal-to-noise (median across all plates, using negative control wells):	-19.02
Signal-to-background (median across all plates, using positive control wells):	2.38
Signal-to-background (median across all plates, using negative control wells):	0.07
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.113480509254699
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.680883055528194

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CEETOX_H295R_TESTO_noMTC_up

Assay Title: Ceetox/OpAns human H295R unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent H295R cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.6975
Neutral control median absolute deviation, by plate:	0.133434
Positive control well median response value, by plate:	6.4325
Positive control well median absolute deviation, by plate:	0.378063
Negative control well median, by plate:	0.712963548693
Negative control well median absolute deviation value, by plate:	0.022239
Z' (median across all plates, using positive control wells):	0.52
Z' (median across all plates, using negative control wells):	0.81
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	-19
Signal-to-noise (median across all plates, using positive control wells):	28.04
Signal-to-noise (median across all plates, using negative control wells):	-19.02
Signal-to-background (median across all plates, using positive control wells):	2.38
Signal-to-background (median across all plates, using negative control wells):	0.07
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.4 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.113480509254699
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.680883055528194

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Ceetox, a part of Cyprotex, is a CRO that in coordination with OpAns, an analytical laboratory, provide ADME-tox services.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB1_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB1_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1789.25
Neutral control median absolute deviation, by plate:	141.588
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.004444595548
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.132994508401286
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB1_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	885
Neutral control median absolute deviation, by plate:	89.3266
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.86692899697034
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.150517867223626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB1_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	965
Neutral control median absolute deviation, by plate:	90.4386
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.2597624368249
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.171299043383423
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB11_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB11_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	84.75
Neutral control median absolute deviation, by plate:	10.3782
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.46423360010208
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.241262756756502
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB11_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	101
Neutral control median absolute deviation, by plate:	18.9031
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.10193039592576
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.279922185832702
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCB11_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	115.5
Neutral control median absolute deviation, by plate:	29.2813
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.18581012446355
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.313575986360194
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCG2_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCG2_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	299.5
Neutral control median absolute deviation, by plate:	38.9182
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.25193048023635
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.204932481101406
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCG2_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	249
Neutral control median absolute deviation, by plate:	32.6172
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.87801500236516
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.245794248762955
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ABCG2_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	177.75
Neutral control median absolute deviation, by plate:	26.6868
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.98408139957195
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.202377865058945
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ACTIN_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ACTIN_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3533.25
Neutral control median absolute deviation, by plate:	401.043
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.658238337343148
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.172058884094211
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ACTIN_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3301
Neutral control median absolute deviation, by plate:	258.714
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.917256804443198
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.18284978145906
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_ACTIN_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	4664.5
Neutral control median absolute deviation, by plate:	400.302
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.0254688027191
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.199171617697287
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A1_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A1_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	80
Neutral control median absolute deviation, by plate:	28.9107
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.92793185536354
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.591699663490599
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A1_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	11.25
Neutral control median absolute deviation, by plate:	6.6717
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	9.13784503044834
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.66

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.653028314193223
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A1_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	11.75
Neutral control median absolute deviation, by plate:	7.78365
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	8.62251297634792
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.68

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.615334596430808
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A2_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A2_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	110.75
Neutral control median absolute deviation, by plate:	25.5748
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.31443701024439
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.440691670003288
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A2_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	54.75
Neutral control median absolute deviation, by plate:	8.8956
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.67973054133036
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.16

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.473054592292178
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP1A2_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	70
Neutral control median absolute deviation, by plate:	21.4977
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.30418352935536
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.353101746564916
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2B6_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2B6_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	52.25
Neutral control median absolute deviation, by plate:	10.3782
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.59893790935123
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.396566308888419
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2B6_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	32
Neutral control median absolute deviation, by plate:	6.6717
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.61199082053125
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.21

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.415288000995156
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2B6_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	46.5
Neutral control median absolute deviation, by plate:	12.9728
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.16057962873147
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.450385337247368
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C9_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C9_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	912.25
Neutral control median absolute deviation, by plate:	270.204
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.67745418082963
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.46

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.31784422965455
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C9_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2430
Neutral control median absolute deviation, by plate:	522.246
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.15122764231805
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.25

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.364082093554195
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C9_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	836.75
Neutral control median absolute deviation, by plate:	277.987
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.03110903199788
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.349259404300352
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C19_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C19_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1282.75
Neutral control median absolute deviation, by plate:	159.009
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.10936441126045
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.194893320602208
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C19_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1448.5
Neutral control median absolute deviation, by plate:	222.761
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.087987665608
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.192221827160702
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP2C19_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1328.5
Neutral control median absolute deviation, by plate:	107.859
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.903458758963849
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.17832417800436
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP3A4_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP3A4_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	20
Neutral control median absolute deviation, by plate:	6.6717
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.62058641045188
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.64

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.56118133263598
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP3A4_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	12
Neutral control median absolute deviation, by plate:	8.8956
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	5.94303918780084
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.75

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.719693813962598
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_CYP3A4_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.5
Neutral control median absolute deviation, by plate:	1.4826
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	6.83825771269529
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.783724870256564
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GAPDH_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GAPDH_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	5253.75
Neutral control median absolute deviation, by plate:	615.279
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.684785744827
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.137058216940566
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GAPDH_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	5726.25
Neutral control median absolute deviation, by plate:	670.506
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.9297607105363
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.160283877217596
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GAPDH_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	5719
Neutral control median absolute deviation, by plate:	442.556
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.642500620474801
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.155549826655433
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GSTA2_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GSTA2_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1459
Neutral control median absolute deviation, by plate:	162.345
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.2259189313796
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.182317330966514
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GSTA2_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	688.75
Neutral control median absolute deviation, by plate:	90.4386
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.14338661103531
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.185081508100509
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_GSTA2_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	218
Neutral control median absolute deviation, by plate:	37.4356
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.13613346403322
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.199606002733914
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_HMGCS2_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_HMGCS2_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	75.5
Neutral control median absolute deviation, by plate:	32.2466
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.4506756579902
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.42

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.524302148911754
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_HMGCS2_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	164
Neutral control median absolute deviation, by plate:	37.065
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.72813457648612
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.26

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.422408161738405
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_HMGCS2_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	370.5
Neutral control median absolute deviation, by plate:	69.3116
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.09193588620171
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.484408535714739
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SLCO1B1_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SLCO1B1_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	444.25
Neutral control median absolute deviation, by plate:	54.4856
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.6589708876469
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.253892670119445
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SLCO1B1_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	401.75
Neutral control median absolute deviation, by plate:	59.304
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.23651688004411
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.21

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.2643970098297
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SLCO1B1_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	493.75
Neutral control median absolute deviation, by plate:	111.936
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.07690391360321
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.248674902440699
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SULT2A_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SULT2A_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	21.25
Neutral control median absolute deviation, by plate:	5.9304
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.13515958328163
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.44412807388402
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SULT2A_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	39.5
Neutral control median absolute deviation, by plate:	7.413
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	4.63594370619387
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.429222510653292
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_SULT2A_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	280.25
Neutral control median absolute deviation, by plate:	40.4008
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.60308663854318
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.384258977273262
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_UGT1A1_0hr_negctrl

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_UGT1A1_6hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	360.5
Neutral control median absolute deviation, by plate:	38.177
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.76038402177095
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.203421751283061
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_UGT1A1_24hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	453.25
Neutral control median absolute deviation, by plate:	30.7639
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	2.48852892328283
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.20905374360745
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

CLD_UGT1A1_48hr

Assay Title: CellzDirect human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

a CRO formerly known as CellzDirect

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	704.75
Neutral control median absolute deviation, by plate:	87.844
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.72933072925689
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	4
Standard minimum concentration tested:	0.00399999999999998 µM
Standard maximum concentration tested:	39.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.187852051437315
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	1

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

a CRO formerly known as CellzDirect

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

HuRELmouseFibroblast_AUC_Con_48h

Assay Title: HuRELmouseFibroblast_AUC_Con_48h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

HuRELmouseFibroblast_AUC_Con_309h

Assay Title: HuRELmouseFibroblast_AUC_Con_309h

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

ACEA Biosciences, Inc. (ACEA) is a privately owned biotechnology company that developed a real-time, label-free, cell-based assay system based on a microelectronic readout called xCELLigence.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCB1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family B (MDR/TAP), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.13386
Neutral control median absolute deviation, by plate:	0.103008
Positive control well median response value, by plate:	2.17062
Positive control well median absolute deviation, by plate:	0.0618867
Negative control well median, by plate:	6.21033363039241
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.42
Z' (median across all plates, using negative control wells):	1
SSMD (median across all plates, using positive control wells):	-6
SSMD (median across all plates, using negative control wells):	-1281
Signal-to-noise (median across all plates, using positive control wells):	-9.45
Signal-to-noise (median across all plates, using negative control wells):	-2793.91
Signal-to-background (median across all plates, using positive control wells):	0.68
Signal-to-background (median across all plates, using negative control wells):	-100.84
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.0299999999999998 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.126465046759354
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.379395140278062

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCB1_up

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family B (MDR/TAP), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	3.13386
Neutral control median absolute deviation, by plate:	0.103008
Positive control well median response value, by plate:	2.17062
Positive control well median absolute deviation, by plate:	0.0618867
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	0
Z' (median across all plates, using positive control wells):	0.42
Z' (median across all plates, using negative control wells):	1
SSMD (median across all plates, using positive control wells):	-6
SSMD (median across all plates, using negative control wells):	-1281
Signal-to-noise (median across all plates, using positive control wells):	-9.45
Signal-to-noise (median across all plates, using negative control wells):	-2793.91
Signal-to-background (median across all plates, using positive control wells):	0.68
Signal-to-background (median across all plates, using negative control wells):	-100.84
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCB11_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family B (MDR/TAP), member 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCB11_up

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family B (MDR/TAP), member 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCC2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family C (CFTR/MRP), member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCC2_up

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family C (CFTR/MRP), member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCC3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family C (CFTR/MRP), member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCC3_up

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family C (CFTR/MRP), member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCG2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family G (WHITE), member 2 (Junior blood group) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ABCG2_up

Assay Title: LifeTech/Expression Analysis human HepaRG ATP-binding cassette, sub-family G (WHITE), member 2 (Junior blood group) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ACLY_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ATP citrate lyase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ACLY_up

Assay Title: LifeTech/Expression Analysis human HepaRG ATP citrate lyase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ACOX1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG acyl-CoA oxidase 1, palmitoyl assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ACOX1_up

Assay Title: LifeTech/Expression Analysis human HepaRG acyl-CoA oxidase 1, palmitoyl assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ADK_dn

Assay Title: LifeTech/Expression Analysis human HepaRG adenosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ADK_up

Assay Title: LifeTech/Expression Analysis human HepaRG adenosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_AFP_dn

Assay Title: LifeTech/Expression Analysis human HepaRG alpha-fetoprotein assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_AFP_up

Assay Title: LifeTech/Expression Analysis human HepaRG alpha-fetoprotein assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ALPP_dn

Assay Title: LifeTech/Expression Analysis human HepaRG alkaline phosphatase, placental assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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GST, Glutathione S-Transferase

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ALPP_up

Assay Title: LifeTech/Expression Analysis human HepaRG alkaline phosphatase, placental assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_APOA5_dn

Assay Title: LifeTech/Expression Analysis human HepaRG apolipoprotein A-V assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_APOA5_up

Assay Title: LifeTech/Expression Analysis human HepaRG apolipoprotein A-V assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BAD_dn

Assay Title: LifeTech/Expression Analysis human HepaRG BCL2-associated agonist of cell death assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BAD_up

Assay Title: LifeTech/Expression Analysis human HepaRG BCL2-associated agonist of cell death assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BAX_dn

Assay Title: LifeTech/Expression Analysis human HepaRG BCL2-associated X protein assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BAX_up

Assay Title: LifeTech/Expression Analysis human HepaRG BCL2-associated X protein assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BCL2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG B-cell CLL/lymphoma 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BCL2_up

Assay Title: LifeTech/Expression Analysis human HepaRG B-cell CLL/lymphoma 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BCL2L11_dn

Assay Title: LifeTech/Expression Analysis human HepaRG BCL2-like 11 (apoptosis facilitator) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BCL2L11_up

Assay Title: LifeTech/Expression Analysis human HepaRG BCL2-like 11 (apoptosis facilitator) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BID_dn

Assay Title: LifeTech/Expression Analysis human HepaRG BH3 interacting domain death agonist assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_BID_up

Assay Title: LifeTech/Expression Analysis human HepaRG BH3 interacting domain death agonist assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CASP3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG caspase 3, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CASP3_up

Assay Title: LifeTech/Expression Analysis human HepaRG caspase 3, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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1.1 Overview

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unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
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Proprietary Elements:

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Caveats:

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unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

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Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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Assay Source Information:

unspecified

References:

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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LTEA_HepaRG_CASP8_up

Assay Title: LifeTech/Expression Analysis human HepaRG caspase 8, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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LTEA_HepaRG_CAT_dn

Assay Title: LifeTech/Expression Analysis human HepaRG catalase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

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unspecified

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Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CCND1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cyclin D1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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LTEA_HepaRG_CCND1_up

Assay Title: LifeTech/Expression Analysis human HepaRG cyclin D1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CCND2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cyclin D2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CCND2_up

Assay Title: LifeTech/Expression Analysis human HepaRG cyclin D2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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Assay Source Information:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CDKN1A_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cyclin-dependent kinase inhibitor 1A (p21, Cip1) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CDKN1A_up

Assay Title: LifeTech/Expression Analysis human HepaRG cyclin-dependent kinase inhibitor 1A (p21, Cip1) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CFLAR_dn

Assay Title: LifeTech/Expression Analysis human HepaRG CASP8 and FADD-like apoptosis regulator assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CFLAR_up

Assay Title: LifeTech/Expression Analysis human HepaRG CASP8 and FADD-like apoptosis regulator assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP1A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 1, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP1A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 1, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP1A2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 1, subfamily A, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP1A2_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 1, subfamily A, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2B6_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily B, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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DMSO, Dimethyl Sulfoxide

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2B6_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily B, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2C8_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily C, polypeptide 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2C8_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily C, polypeptide 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2C9_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily C, polypeptide 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2C9_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily C, polypeptide 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
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Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2C19_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily C, polypeptide 19 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2C19_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily C, polypeptide 19 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2E1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily E, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP2E1_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 2, subfamily E, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP3A4_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 3, subfamily A, polypeptide 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP3A4_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 3, subfamily A, polypeptide 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP3A5_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 3, subfamily A, polypeptide 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP3A5_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 3, subfamily A, polypeptide 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP3A7_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 3, subfamily A, polypeptide 7 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Positive control well median response value, by plate:	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP3A7_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 3, subfamily A, polypeptide 7 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP4A11_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 4, subfamily A, polypeptide 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP4A11_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 4, subfamily A, polypeptide 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP4A22_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 4, subfamily A, polypeptide 22 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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LTEA_HepaRG_CYP4A22_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 4, subfamily A, polypeptide 22 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP7A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 7, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP7A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 7, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP24A1_1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 24, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP24A1_1_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 24, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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CV, Coefficient of Variation

CYP, Cytochrome P450s

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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LTEA_HepaRG_CYP24A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 24, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_CYP24A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG cytochrome P450, family 24, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_DDIT3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG DNA-damage-inducible transcript 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_DDIT3_up

Assay Title: LifeTech/Expression Analysis human HepaRG DNA-damage-inducible transcript 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_EGF_dn

Assay Title: LifeTech/Expression Analysis human HepaRG epidermal growth factor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_EGF_up

Assay Title: LifeTech/Expression Analysis human HepaRG epidermal growth factor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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4.3 Assay Documentation Source

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_EGR1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG early growth response 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_EGR1_up

Assay Title: LifeTech/Expression Analysis human HepaRG early growth response 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_EZR_dn

Assay Title: LifeTech/Expression Analysis human HepaRG ezrin assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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SULT, Sulfotransferases

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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LTEA_HepaRG_EZR_up

Assay Title: LifeTech/Expression Analysis human HepaRG ezrin assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FABP1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG fatty acid binding protein 1, liver assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FABP1_up

Assay Title: LifeTech/Expression Analysis human HepaRG fatty acid binding protein 1, liver assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FAS_dn

Assay Title: LifeTech/Expression Analysis human HepaRG Fas cell surface death receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FAS_up

Assay Title: LifeTech/Expression Analysis human HepaRG Fas cell surface death receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FASN_dn

Assay Title: LifeTech/Expression Analysis human HepaRG fatty acid synthase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FASN_up

Assay Title: LifeTech/Expression Analysis human HepaRG fatty acid synthase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FMO3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG flavin containing monooxygenase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FMO3_up

Assay Title: LifeTech/Expression Analysis human HepaRG flavin containing monooxygenase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FOXO1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG forkhead box O1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FOXO1_up

Assay Title: LifeTech/Expression Analysis human HepaRG forkhead box O1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FOXO3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG forkhead box O3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_FOXO3_up

Assay Title: LifeTech/Expression Analysis human HepaRG forkhead box O3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GADD45A_dn

Assay Title: LifeTech/Expression Analysis human HepaRG growth arrest and DNA-damage-inducible, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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LTEA_HepaRG_GADD45A_up

Assay Title: LifeTech/Expression Analysis human HepaRG growth arrest and DNA-damage-inducible, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GADD45B_dn

Assay Title: LifeTech/Expression Analysis human HepaRG growth arrest and DNA-damage-inducible, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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LTEA_HepaRG_GADD45B_up

Assay Title: LifeTech/Expression Analysis human HepaRG growth arrest and DNA-damage-inducible, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GADD45G_dn

Assay Title: LifeTech/Expression Analysis human HepaRG growth arrest and DNA-damage-inducible, gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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LTEA_HepaRG_GADD45G_up

Assay Title: LifeTech/Expression Analysis human HepaRG growth arrest and DNA-damage-inducible, gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GCLC_dn

Assay Title: LifeTech/Expression Analysis human HepaRG glutamate-cysteine ligase, catalytic subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GCLC_up

Assay Title: LifeTech/Expression Analysis human HepaRG glutamate-cysteine ligase, catalytic subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GCLM_dn

Assay Title: LifeTech/Expression Analysis human HepaRG glutamate-cysteine ligase, modifier subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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SULT, Sulfotransferases

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4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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LTEA_HepaRG_GCLM_up

Assay Title: LifeTech/Expression Analysis human HepaRG glutamate-cysteine ligase, modifier subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GE_ACTB_dn

Assay Title: LifeTech/Expression Analysis human HepaRG actin, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GE_ACTB_up

Assay Title: LifeTech/Expression Analysis human HepaRG actin, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GE_GAPDH_dn

Assay Title: LifeTech/Expression Analysis human HepaRG glyceraldehyde-3-phosphate dehydrogenase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GE_GAPDH_up

Assay Title: LifeTech/Expression Analysis human HepaRG glyceraldehyde-3-phosphate dehydrogenase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GE_POLR2A_dn

Assay Title: LifeTech/Expression Analysis human HepaRG polymerase (RNA) II (DNA directed) polypeptide A, 220kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

unspecified

References:

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GE_POLR2A_up

Assay Title: LifeTech/Expression Analysis human HepaRG polymerase (RNA) II (DNA directed) polypeptide A, 220kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GSTA2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG glutathione S-transferase alpha 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GSTA2_up

Assay Title: LifeTech/Expression Analysis human HepaRG glutathione S-transferase alpha 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GSTM3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG glutathione S-transferase mu 3 (brain) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_GSTM3_up

Assay Title: LifeTech/Expression Analysis human HepaRG glutathione S-transferase mu 3 (brain) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HGF_dn

Assay Title: LifeTech/Expression Analysis human HepaRG hepatocyte growth factor (hepapoietin A; scatter factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HGF_up

Assay Title: LifeTech/Expression Analysis human HepaRG hepatocyte growth factor (hepapoietin A; scatter factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HIF1A_dn

Assay Title: LifeTech/Expression Analysis human HepaRG hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HIF1A_up

Assay Title: LifeTech/Expression Analysis human HepaRG hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HMGCS2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HMGCS2_up

Assay Title: LifeTech/Expression Analysis human HepaRG 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HSPA1A_dn

Assay Title: LifeTech/Expression Analysis human HepaRG heat shock 70kDa protein 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_HSPA1A_up

Assay Title: LifeTech/Expression Analysis human HepaRG heat shock 70kDa protein 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ICAM1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG intercellular adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_ICAM1_up

Assay Title: LifeTech/Expression Analysis human HepaRG intercellular adhesion molecule 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IGF1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG insulin-like growth factor 1 (somatomedin C) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IGF1_up

Assay Title: LifeTech/Expression Analysis human HepaRG insulin-like growth factor 1 (somatomedin C) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IGFBP1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG insulin-like growth factor binding protein 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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DMSO, Dimethyl Sulfoxide

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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LTEA_HepaRG_IGFBP1_up

Assay Title: LifeTech/Expression Analysis human HepaRG insulin-like growth factor binding protein 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IL6_dn

Assay Title: LifeTech/Expression Analysis human HepaRG interleukin 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IL6_up

Assay Title: LifeTech/Expression Analysis human HepaRG interleukin 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IL6R_dn

Assay Title: LifeTech/Expression Analysis human HepaRG interleukin 6 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_IL6R_up

Assay Title: LifeTech/Expression Analysis human HepaRG interleukin 6 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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Assay Source Information:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_JUN_dn

Assay Title: LifeTech/Expression Analysis human HepaRG jun proto-oncogene assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_JUN_up

Assay Title: LifeTech/Expression Analysis human HepaRG jun proto-oncogene assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_KCNK1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG potassium channel, subfamily K, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_KCNK1_up

Assay Title: LifeTech/Expression Analysis human HepaRG potassium channel, subfamily K, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_KLK3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG kallikrein-related peptidase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_KLK3_up

Assay Title: LifeTech/Expression Analysis human HepaRG kallikrein-related peptidase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_KRT19_dn

Assay Title: LifeTech/Expression Analysis human HepaRG keratin 19 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_KRT19_up

Assay Title: LifeTech/Expression Analysis human HepaRG keratin 19 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_LDH_cytotoxicity

Assay Title: LifeTech/Expression Analysis human HepaRG unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_LIPC_dn

Assay Title: LifeTech/Expression Analysis human HepaRG lipase, hepatic assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_LIPC_up

Assay Title: LifeTech/Expression Analysis human HepaRG lipase, hepatic assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_LPL_dn

Assay Title: LifeTech/Expression Analysis human HepaRG lipoprotein lipase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_LPL_up

Assay Title: LifeTech/Expression Analysis human HepaRG lipoprotein lipase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MIR122_dn

Assay Title: LifeTech/Expression Analysis human HepaRG microRNA 122 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MIR122_up

Assay Title: LifeTech/Expression Analysis human HepaRG microRNA 122 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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LTEA_HepaRG_MMP1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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1.3 Assay References

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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SSMD (median across all plates, using negative control wells):	unspecified
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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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EDC, Endocrine disrupting chemicals

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Assay Title: LifeTech/Expression Analysis human HepaRG matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MMP3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG matrix metallopeptidase 3 (stromelysin 1, progelatinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

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3.2 Assay Performance

Assay Performance Measures:

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Analytical Elements:

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the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MMP10_dn

Assay Title: LifeTech/Expression Analysis human HepaRG matrix metallopeptidase 10 (stromelysin 2) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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RTCA, Real-Time Cell Analysis

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MMP10_up

Assay Title: LifeTech/Expression Analysis human HepaRG matrix metallopeptidase 10 (stromelysin 2) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MYC_dn

Assay Title: LifeTech/Expression Analysis human HepaRG v-myc avian myelocytomatosis viral oncogene homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_MYC_up

Assay Title: LifeTech/Expression Analysis human HepaRG v-myc avian myelocytomatosis viral oncogene homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_NFE2L2_dn

Assay Title: LifeTech/Expression Analysis human HepaRG nuclear factor, erythroid 2-like 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_NFE2L2_up

Assay Title: LifeTech/Expression Analysis human HepaRG nuclear factor, erythroid 2-like 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_NFKB1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_NFKB1_up

Assay Title: LifeTech/Expression Analysis human HepaRG nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_NQO1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG NAD(P)H dehydrogenase, quinone 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_NQO1_up

Assay Title: LifeTech/Expression Analysis human HepaRG NAD(P)H dehydrogenase, quinone 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PDK4_dn

Assay Title: LifeTech/Expression Analysis human HepaRG pyruvate dehydrogenase kinase, isozyme 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PDK4_up

Assay Title: LifeTech/Expression Analysis human HepaRG pyruvate dehydrogenase kinase, isozyme 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PEG10_dn

Assay Title: LifeTech/Expression Analysis human HepaRG paternally expressed 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PEG10_up

Assay Title: LifeTech/Expression Analysis human HepaRG paternally expressed 10 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PPP2R4_dn

Assay Title: LifeTech/Expression Analysis human HepaRG protein phosphatase 2A activator, regulatory subunit 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PPP2R4_up

Assay Title: LifeTech/Expression Analysis human HepaRG protein phosphatase 2A activator, regulatory subunit 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PTEN_dn

Assay Title: LifeTech/Expression Analysis human HepaRG phosphatase and tensin homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_PTEN_up

Assay Title: LifeTech/Expression Analysis human HepaRG phosphatase and tensin homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SDHB_dn

Assay Title: LifeTech/Expression Analysis human HepaRG succinate dehydrogenase complex, subunit B, iron sulfur (Ip) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SDHB_up

Assay Title: LifeTech/Expression Analysis human HepaRG succinate dehydrogenase complex, subunit B, iron sulfur (Ip) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLC10A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier family 10 (sodium/bile acid cotransporter), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLC10A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier family 10 (sodium/bile acid cotransporter), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLC22A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier family 22 (organic cation transporter), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLC22A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier family 22 (organic cation transporter), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLC22A6_dn

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier family 22 (organic anion transporter), member 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLC22A6_up

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier family 22 (organic anion transporter), member 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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NCI, Normalized Cell Index

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SULT, Sulfotransferases

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLCO1B1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier organic anion transporter family, member 1B1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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unspecified

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SLCO1B1_up

Assay Title: LifeTech/Expression Analysis human HepaRG solute carrier organic anion transporter family, member 1B1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_STAT3_dn

Assay Title: LifeTech/Expression Analysis human HepaRG signal transducer and activator of transcription 3 (acute-phase response factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_STAT3_up

Assay Title: LifeTech/Expression Analysis human HepaRG signal transducer and activator of transcription 3 (acute-phase response factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_SULT2A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

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EPA NCCT

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LTEA_HepaRG_SULT2A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TGFA_dn

Assay Title: LifeTech/Expression Analysis human HepaRG transforming growth factor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TGFA_up

Assay Title: LifeTech/Expression Analysis human HepaRG transforming growth factor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TGFB1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TGFB1_up

Assay Title: LifeTech/Expression Analysis human HepaRG transforming growth factor, beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TIMP1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG TIMP metallopeptidase inhibitor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TIMP1_up

Assay Title: LifeTech/Expression Analysis human HepaRG TIMP metallopeptidase inhibitor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TNFRSF1A_dn

Assay Title: LifeTech/Expression Analysis human HepaRG tumor necrosis factor receptor superfamily, member 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TNFRSF1A_up

Assay Title: LifeTech/Expression Analysis human HepaRG tumor necrosis factor receptor superfamily, member 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TP53_dn

Assay Title: LifeTech/Expression Analysis human HepaRG tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_TP53_up

Assay Title: LifeTech/Expression Analysis human HepaRG tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_UGT1A1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG UDP glucuronosyltransferase 1 family, polypeptide A1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_UGT1A1_up

Assay Title: LifeTech/Expression Analysis human HepaRG UDP glucuronosyltransferase 1 family, polypeptide A1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_UGT1A6_dn

Assay Title: LifeTech/Expression Analysis human HepaRG UDP glucuronosyltransferase 1 family, polypeptide A6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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LTEA_HepaRG_UGT1A6_up

Assay Title: LifeTech/Expression Analysis human HepaRG UDP glucuronosyltransferase 1 family, polypeptide A6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

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1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_XBP1_dn

Assay Title: LifeTech/Expression Analysis human HepaRG X-box binding protein 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

LTEA_HepaRG_XBP1_up

Assay Title: LifeTech/Expression Analysis human HepaRG X-box binding protein 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent HepaRG cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HEK293T_CellTiterGLO

Assay Title: NCCT"s Simmons Lab human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	477985
Neutral control median absolute deviation, by plate:	25838.4
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.8064189805534
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.60777764752233
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

Paul KB, Hedge JM, Rotroff DM, Hornung MW, Crofton KM, Simmons SO. Development of a thyroperoxidase inhibition assay for high-throughput screening. Chem Res Toxicol. 2014 Mar 17;27(3):387-99. doi: 10.1021/tx400310w. Epub 2014 Jan 14. PubMed PMID: 24383450.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Apoptosis_DMEM_6hr

Assay Title: NCCT_HTTr_Apoptosis_DMEM_6hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Apoptosis_DMEM_12hr

Assay Title: NCCT_HTTr_Apoptosis_DMEM_12hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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NCCT_HTTr_Apoptosis_DMEM_24hr

Assay Title: NCCT_HTTr_Apoptosis_DMEM_24hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

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Date of Assay Document Creation:

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EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Apoptosis_PRF_DMEM_6hr

Assay Title: NCCT_HTTr_Apoptosis_PRF_DMEM_6hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

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NCCT_HTTr_Apoptosis_PRF_DMEM_12hr

Assay Title: NCCT_HTTr_Apoptosis_PRF_DMEM_12hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Assay Title: NCCT_HTTr_Apoptosis_PRF_DMEM_24hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Viability_DMEM_6hr

Assay Title: NCCT_HTTr_Viability_DMEM_6hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Viability_DMEM_12hr

Assay Title: NCCT_HTTr_Viability_DMEM_12hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Viability_DMEM_24hr

Assay Title: NCCT_HTTr_Viability_DMEM_24hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Viability_PRF_DMEM_6hr

Assay Title: NCCT_HTTr_Viability_PRF_DMEM_6hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Viability_PRF_DMEM_12hr

Assay Title: NCCT_HTTr_Viability_PRF_DMEM_12hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_HTTr_Viability_PRF_DMEM_24hr

Assay Title: NCCT_HTTr_Viability_PRF_DMEM_24hr

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_basal_resp_rate_OCR_dn

Assay Title: NCCT_MITO_basal_resp_rate_OCR_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_basal_resp_rate_OCR_up

Assay Title: NCCT_MITO_basal_resp_rate_OCR_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_inhib_resp_rate_OCR_dn

Assay Title: NCCT_MITO_inhib_resp_rate_OCR_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_inhib_resp_rate_OCR_up

Assay Title: NCCT_MITO_inhib_resp_rate_OCR_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_max_resp_rate_OCR_dn

Assay Title: NCCT_MITO_max_resp_rate_OCR_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_max_resp_rate_OCR_up

Assay Title: NCCT_MITO_max_resp_rate_OCR_up

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_MITO_viability

Assay Title: NCCT_MITO_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_QuantiLum_inhib_2_dn

Assay Title: NCCT_QuantiLum_inhib_2_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1026250
Neutral control median absolute deviation, by plate:	43824.2
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.8542121344391
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.02867425089386
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_QuantiLum_inhib_dn

Assay Title: NCCT_QuantiLum_inhib_dn

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	37834.5
Neutral control median absolute deviation, by plate:	1418.85
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.5107367248677
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.09644317588144
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.2893295276443

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

Paul KB, Hedge JM, Rotroff DM, Hornung MW, Crofton KM, Simmons SO. Development of a thyroperoxidase inhibition assay for high-throughput screening. Chem Res Toxicol. 2014 Mar 17;27(3):387-99. doi: 10.1021/tx400310w. Epub 2014 Jan 14. PubMed PMID: 24383450.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_TPO_AUR_dn

Assay Title: NCCT"s Simmons Lab rat unspecified thyroid peroxidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	51492.5
Neutral control median absolute deviation, by plate:	2226.87
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.710141537022
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.089999999999999 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.55295187687267
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

Paul KB, Hedge JM, Rotroff DM, Hornung MW, Crofton KM, Simmons SO. Development of a thyroperoxidase inhibition assay for high-throughput screening. Chem Res Toxicol. 2014 Mar 17;27(3):387-99. doi: 10.1021/tx400310w. Epub 2014 Jan 14. PubMed PMID: 24383450.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NCCT_TPO_GUA_dn

Assay Title: NCCT"s Simmons Lab pig unspecified thyroid peroxidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NCCT"s Simmons Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.40725
Neutral control median absolute deviation, by plate:	0.007413
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.909338168631
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.0500000000000001 µM
Standard maximum concentration tested:	500.000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.7084553570984
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NCCT"s Simmons Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_GSC_CellCount_dn

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_GSC_CellCount_up

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_GSC_GSCvCellCount_dn

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_GSC_GSCvCellCount_up

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MEA_AB

Assay Title: NHEERL SHAFER LAB MEA rat primary cortical cell culture unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

adherent primary cortical cell culture primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL SHAFER LAB MEA

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL SHAFER LAB MEA

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MEA_LDH

Assay Title: NHEERL SHAFER LAB MEA rat primary cortical cell culture unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

adherent primary cortical cell culture primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL SHAFER LAB MEA

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL SHAFER LAB MEA

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MEA_MFR_dn

Assay Title: NHEERL SHAFER LAB MEA rat primary cortical cell culture unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

adherent primary cortical cell culture primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL SHAFER LAB MEA

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL SHAFER LAB MEA

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MEA_MFR_up

Assay Title: NHEERL SHAFER LAB MEA rat primary cortical cell culture unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

adherent primary cortical cell culture primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL SHAFER LAB MEA

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL SHAFER LAB MEA

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MHC_CellCount_dn

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1
Neutral control median absolute deviation, by plate:	0.0828999
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	7.82882821991901
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.01 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.156745049125398
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.470235147376194

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MHC_CellCount_up

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	1
Neutral control median absolute deviation, by plate:	0.0828999
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	1.30118777660075
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.01 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.147704979004369
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.443114937013107

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MHC_MHCvCellCount_dn

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.999886
Neutral control median absolute deviation, by plate:	0.0675383
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	3.62441915756425
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.01 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.130880228271957
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.392640684815871

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_MHC_MHCvCellCount_up

Assay Title: NHEERL"s Hunter Lab mouse J1 embryonic stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

adherent J1 embryonic stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Hunter Lab

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.999886
Neutral control median absolute deviation, by plate:	0.0675383
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	0.571969320027072
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	4
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.01 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.12680549652734
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	0.38041648958202

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Hunter Lab

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NHEERL_ZF_144hpf_TERATOSCORE_up

Assay Title: National Health and Environmental Effects Research Lab - Padilla Lab zebrafish zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL"s Padilla laboratory focuses on the development and screening of Zebrafish assays

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	2.03048
Neutral control median absolute deviation, by plate:	1.15304
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.9588803033427
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.58

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.0299999999999998 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.53481347591287
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL"s Padilla laboratory focuses on the development and screening of Zebrafish assays

References:

Padilla S, Corum D, Padnos B, Hunter DL, Beam A, Houck KA, Sipes N, Kleinstreuer N, Knudsen T, Dix DJ, Reif DM. Zebrafish developmental screening of the ToxCastâ„¢ Phase I chemical library. Reprod Toxicol. 2012 Apr;33(2):174-87. doi: 10.1016/j.reprotox.2011.10.018. Epub 2011 Dec 9. PubMed PMID: 22182468.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NIS_HEK293T_CTG_Cytotoxicity

Assay Title: NHEERL Stoker and Laws Laboratories human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL Stoker and Laws Laboratories

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

A novel cell line expressing human NIS, hNIS-HEK293T-EPA, was used in a radioactive iodide uptake (RAIU) assay to identify inhibitors of NIS-mediated iodide uptake. A parallel cytoxicity assay (Cell Titer Glo, Promega) was also run in the same cell line.

Assay Quality Statistics:

Neutral control well median response value, by plate:	51712.2
Neutral control median absolute deviation, by plate:	1820.26
Positive control well median response value, by plate:	49606
Positive control well median absolute deviation, by plate:	2848.45
Negative control well median, by plate:	95.4937384520632
Negative control well median absolute deviation value, by plate:	1816.18
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	0
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	0
Signal-to-noise (median across all plates, using positive control wells):	-0.8
Signal-to-noise (median across all plates, using negative control wells):	-0.66
Signal-to-background (median across all plates, using positive control wells):	0.97
Signal-to-background (median across all plates, using negative control wells):	0.98
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.32969410220538
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	15.9890823066161

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL Stoker and Laws Laboratories

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NIS_RAIU_inhibition

Assay Title: NHEERL Stoker and Laws Laboratories human HEK293T solute carrier family 5 member 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NHEERL Stoker and Laws Laboratories

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

A novel cell line expressing human NIS, hNIS-HEK293T-EPA, was used in a radioactive iodide uptake (RAIU) assay to identify inhibitors of NIS-mediated iodide uptake. A parallel cytoxicity assay (Cell Titer Glo, Promega) was also run in the same cell line.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19062.5
Neutral control median absolute deviation, by plate:	1505.58
Positive control well median response value, by plate:	1003
Positive control well median absolute deviation, by plate:	639.001
Negative control well median, by plate:	96.9095744680851
Negative control well median absolute deviation value, by plate:	462.571
Z' (median across all plates, using positive control wells):	0.68
Z' (median across all plates, using negative control wells):	0
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	-1
Signal-to-noise (median across all plates, using positive control wells):	-12.12
Signal-to-noise (median across all plates, using negative control wells):	-1.16
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	0.91
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.92409496696615
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.7722849008984

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NHEERL Stoker and Laws Laboratories

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP1A1

Assay Title: Novascreen human unspecified cytochrome P450, family 1, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP1A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP1A1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP1A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP1A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP1A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP1A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	41633
Neutral control median absolute deviation, by plate:	535.219
Positive control well median response value, by plate:	13842
Positive control well median absolute deviation, by plate:	60.7866
Negative control well median, by plate:	103.544144024447
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-46
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-46.4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.8911470059282
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP1A1_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 1, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP1A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP1A1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP1A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP1A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP1A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP1A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	41633
Neutral control median absolute deviation, by plate:	535.219
Positive control well median response value, by plate:	13842
Positive control well median absolute deviation, by plate:	60.7866
Negative control well median, by plate:	21.1265716038025
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-46
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-46.4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.8911470059282
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP1A2

Assay Title: Novascreen human unspecified cytochrome P450, family 1, subfamily A, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP1A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP1A2, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP1A2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP1A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP1A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP1A2 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	146423
Neutral control median absolute deviation, by plate:	2121.6
Positive control well median response value, by plate:	24271
Positive control well median absolute deviation, by plate:	461.089
Negative control well median, by plate:	111.15107612778
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-62
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.22140528143636
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP1A2_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 1, subfamily A, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP1A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP1A2_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP1A2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP1A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP1A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP1A2 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	146423
Neutral control median absolute deviation, by plate:	2121.6
Positive control well median response value, by plate:	24271
Positive control well median absolute deviation, by plate:	461.089
Negative control well median, by plate:	25.2554213606685
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-62
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.22140528143636
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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SSMD, Strictly Standardized Mean Difference

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP1B1

Assay Title: Novascreen human unspecified cytochrome P450, family 1, subfamily B, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP1B1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP1B1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP1B1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP1B1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP1B1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP1B1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35215
Neutral control median absolute deviation, by plate:	265.385
Positive control well median response value, by plate:	9876
Positive control well median absolute deviation, by plate:	300.226
Negative control well median, by plate:	94.2296845351044
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-47
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-141.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.22810294845157
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.3686176907094

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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NVS_ADME_hCYP1B1_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 1, subfamily B, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP1B1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP1B1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP1B1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP1B1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP1B1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

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Negative control well median absolute deviation value, by plate:	unspecified
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Z’ (median across all plates, using negative control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

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Biological Response:

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Analytical Elements:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2A6

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily A, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2A6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2A6, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2A6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2A6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2A6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2A6 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7744
Neutral control median absolute deviation, by plate:	87.4734
Positive control well median response value, by plate:	2228
Positive control well median absolute deviation, by plate:	84.5082
Negative control well median, by plate:	91.649031417874
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-67.68
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.29225074962519
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2A6_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily A, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2A6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2A6_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2A6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2A6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2A6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

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Assay Objectives:

NVS_ADME_hCYP2A6 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7744
Neutral control median absolute deviation, by plate:	87.4734
Positive control well median response value, by plate:	2228
Positive control well median absolute deviation, by plate:	84.5082
Negative control well median, by plate:	28.1849834836175
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-67.68
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.29225074962519
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2B6

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily B, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2B6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2B6, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2B6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2B6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2B6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2B6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26844.5
Neutral control median absolute deviation, by plate:	260.196
Positive control well median response value, by plate:	8620.5
Positive control well median absolute deviation, by plate:	71.1648
Negative control well median, by plate:	126.047097920128
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-71
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-78.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.33
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.55763530034319
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.1 Resources

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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NVS_ADME_hCYP2B6_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily B, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2B6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2B6_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2B6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2B6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2B6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

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Assay Objectives:

NVS_ADME_hCYP2B6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	260.196
Positive control well median response value, by plate:	8620.5
Positive control well median absolute deviation, by plate:	71.1648
Negative control well median, by plate:	22.8621182909385
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-71
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-78.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.33
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.55763530034319
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C8

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C8 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C8, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C8, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C8 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C8 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28514.5
Neutral control median absolute deviation, by plate:	1046.72
Positive control well median response value, by plate:	1178.5
Positive control well median absolute deviation, by plate:	16.3086
Negative control well median, by plate:	99.71713898537
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-38
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-38.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.82134772441714
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.9280863465028

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C8_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 8 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C8 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C8_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C8, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C8 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C8 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28514.5
Neutral control median absolute deviation, by plate:	1046.72
Positive control well median response value, by plate:	1178.5
Positive control well median absolute deviation, by plate:	16.3086
Negative control well median, by plate:	19.2761412815859
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-38
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-38.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.82134772441714
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.9280863465028

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C9

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C9 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C9, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C9, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C9 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C9 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13062
Neutral control median absolute deviation, by plate:	155.673
Positive control well median response value, by plate:	3118.5
Positive control well median absolute deviation, by plate:	48.9258
Negative control well median, by plate:	4507.25128813625
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-67
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-73.41
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	8
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.19125446248241
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C9_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C9 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C9_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C9, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C9 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C9 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13062
Neutral control median absolute deviation, by plate:	155.673
Positive control well median response value, by plate:	3118.5
Positive control well median absolute deviation, by plate:	48.9258
Negative control well median, by plate:	466.352973969994
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-67
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-73.41
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.19125446248241
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C18

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 18 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C18 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C18, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C18. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C18, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C18 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C18 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	12109.5
Neutral control median absolute deviation, by plate:	228.32
Positive control well median response value, by plate:	1368
Positive control well median absolute deviation, by plate:	27.4281
Negative control well median, by plate:	101.383069784205
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-44
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.2402555060755
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C18_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 18 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C18 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C18_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C18. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C18, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C18 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C18 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	12109.5
Neutral control median absolute deviation, by plate:	228.32
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Negative control well median, by plate:	7.20642290578448
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Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-44
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Standard minimum concentration tested:	0.0089999999999999 µM
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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C19

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 19 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C19 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C19, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C19. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C19, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C19 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C19 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	108354
Neutral control median absolute deviation, by plate:	919.953
Positive control well median response value, by plate:	19699
Positive control well median absolute deviation, by plate:	197.186
Negative control well median, by plate:	520.96854234622
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-98
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-110.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.60299124232151
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2C19_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily C, polypeptide 19 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2C19 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2C19_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2C19. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2C19, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2C19 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2C19 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	108354
Neutral control median absolute deviation, by plate:	919.953
Positive control well median response value, by plate:	19699
Positive control well median absolute deviation, by plate:	197.186
Negative control well median, by plate:	114.750022069858
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-98
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-110.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.60299124232151
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2D6

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily D, polypeptide 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2D6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2D6, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2D6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2D6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2D6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2D6 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10667.5
Neutral control median absolute deviation, by plate:	104.523
Positive control well median response value, by plate:	3498
Positive control well median absolute deviation, by plate:	38.5476
Negative control well median, by plate:	557.798852169161
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-84.69
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Nominal number of tested concentrations:	8
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Chemical Library Scope and Limitations:

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Date of Assay Document Creation:

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NVS_ADME_hCYP2D6_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily D, polypeptide 6 assay

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1.1 Overview

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1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
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Proprietary Elements:

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Caveats:

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Status:

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None reported.

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Negative control well median, by plate:	51.3433868510602
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-84.69
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.39070661107565
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2E1

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily E, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2E1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2E1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2E1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2E1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2E1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2E1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30877
Neutral control median absolute deviation, by plate:	547.821
Positive control well median response value, by plate:	4867.5
Positive control well median absolute deviation, by plate:	35.5824
Negative control well median, by plate:	90.4419841986088
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-473
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-474.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.01545010548218
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Assay Source Information:

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2E1_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily E, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2E1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2E1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2E1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2E1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2E1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2E1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30877
Neutral control median absolute deviation, by plate:	547.821
Positive control well median response value, by plate:	4867.5
Positive control well median absolute deviation, by plate:	35.5824
Negative control well median, by plate:	7.49572094670365
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-473
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-474.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.01545010548218
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2J2

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily J, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2J2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2J2, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2J2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2J2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2J2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2J2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13755
Neutral control median absolute deviation, by plate:	119.349
Positive control well median response value, by plate:	1815.5
Positive control well median absolute deviation, by plate:	79.3191
Negative control well median, by plate:	122.239643922917
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-76
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-154.06
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.69987482675877
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.1992489605526

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP2J2_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 2, subfamily J, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP2J2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP2J2_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP2J2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP2J2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP2J2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP2J2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13755
Neutral control median absolute deviation, by plate:	119.349
Positive control well median response value, by plate:	1815.5
Positive control well median absolute deviation, by plate:	79.3191
Negative control well median, by plate:	47.3587372294582
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-76
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-154.06
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.69987482675877
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.1992489605526

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP3A4

Assay Title: Novascreen human unspecified cytochrome P450, family 3, subfamily A, polypeptide 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP3A4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP3A4, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP3A4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP3A4, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP3A4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP3A4 is a biochemical, single-readout assay that uses extracted gene-proteins from insect cells in a cell-free assay.Measurements were taken 0.33 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36291.5
Neutral control median absolute deviation, by plate:	170.499
Positive control well median response value, by plate:	4124
Positive control well median absolute deviation, by plate:	54.8562
Negative control well median, by plate:	8910.83096714883
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-101
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-134.93
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.85687847312388
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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NVS_ADME_hCYP3A4_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 3, subfamily A, polypeptide 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP3A4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP3A4_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP3A4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP3A4, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP3A4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP3A5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP3A5, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP3A5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP3A5, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP3A5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP3A5 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	11771
Neutral control median absolute deviation, by plate:	51.891
Positive control well median response value, by plate:	3467
Positive control well median absolute deviation, by plate:	237.216
Negative control well median, by plate:	88.0867072733787
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-160.03
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.584328197778574
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP3A5_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 3, subfamily A, polypeptide 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP3A5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP3A5_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP3A5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP3A5, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP3A5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP3A5 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	11771
Neutral control median absolute deviation, by plate:	51.891
Positive control well median response value, by plate:	3467
Positive control well median absolute deviation, by plate:	237.216
Negative control well median, by plate:	0.806162665711215
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-160.03
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.584328197778574
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP4F12

Assay Title: Novascreen human unspecified cytochrome P450, family 4, subfamily F, polypeptide 12 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP4F12 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP4F12, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP4F12. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP4F12, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP4F12 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP4F12 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26290.5
Neutral control median absolute deviation, by plate:	359.53
Positive control well median response value, by plate:	7041.5
Positive control well median absolute deviation, by plate:	57.0801
Negative control well median, by plate:	98.0178035400062
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.28
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.98302163652091
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP4F12_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 4, subfamily F, polypeptide 12 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP4F12 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP4F12_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP4F12. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP4F12, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP4F12 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP4F12 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

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Positive control well median absolute deviation, by plate:	57.0801
Negative control well median, by plate:	380.096308186196
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.28
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.98302163652091
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP19A1

Assay Title: Novascreen human unspecified cytochrome P450, family 19, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP19A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP19A1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP19A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "steroidogenesis-related".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP19A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP19A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP19A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	11984
Neutral control median absolute deviation, by plate:	51.891
Positive control well median response value, by plate:	4216.5
Positive control well median absolute deviation, by plate:	25.2042
Negative control well median, by plate:	100.756347920043
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-94
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-158.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.37
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.41846923010393
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_hCYP19A1_Activator

Assay Title: Novascreen human unspecified cytochrome P450, family 19, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_hCYP19A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_hCYP19A1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CYP19A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "steroidogenesis-related". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_hCYP19A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_hCYP19A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_hCYP19A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	11984
Neutral control median absolute deviation, by plate:	51.891
Positive control well median response value, by plate:	4216.5
Positive control well median absolute deviation, by plate:	25.2042
Negative control well median, by plate:	27.4377529743653
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-94
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-158.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.37
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.41846923010393
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP1A1

Assay Title: Novascreen rat unspecified cytochrome P450, family 1, subfamily a, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP1A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP1A1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp1a1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP1A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP1A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP1A1 is a biochemical, single-readout assay that uses extracted gene-proteins from baculovirus-insect cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32962
Neutral control median absolute deviation, by plate:	979.257
Positive control well median response value, by plate:	7071.5
Positive control well median absolute deviation, by plate:	539.666
Negative control well median, by plate:	86.115646310666
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-31.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

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Nominal number of tested concentrations:	8
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.19248820443626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP1A1_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 1, subfamily a, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP1A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP1A1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp1a1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP1A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP1A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP1A1 is a biochemical, single-readout assay that uses extracted gene-proteins from baculovirus-insect cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32962
Neutral control median absolute deviation, by plate:	979.257
Positive control well median response value, by plate:	7071.5
Positive control well median absolute deviation, by plate:	539.666
Negative control well median, by plate:	22.4822466107166
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-31.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.19248820443626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP1A2

Assay Title: Novascreen rat unspecified cytochrome P450, family 1, subfamily a, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP1A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP1A2, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp1a2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP1A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP1A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP1A2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	170624
Neutral control median absolute deviation, by plate:	1119.36
Positive control well median response value, by plate:	99999
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	492.301474662495
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-116
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-132.6
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.00500000000000001 µM
Standard maximum concentration tested:	10 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	21.751519026778
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	130.509114160668

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP1A2_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 1, subfamily a, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP1A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP1A2_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp1a2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP1A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP1A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP1A2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	170624
Neutral control median absolute deviation, by plate:	1119.36
Positive control well median response value, by plate:	99999
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	1186.7800281621
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-116
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-132.6
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Nominal number of tested concentrations:	8
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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2A1

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily a, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2A1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2a1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32547
Neutral control median absolute deviation, by plate:	318.759
Positive control well median response value, by plate:	6108
Positive control well median absolute deviation, by plate:	96.369
Negative control well median, by plate:	101.50202520252
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-68
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-82.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.34941708254929
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2A1_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily a, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2A1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2a1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32547
Neutral control median absolute deviation, by plate:	318.759
Positive control well median response value, by plate:	6108
Positive control well median absolute deviation, by plate:	96.369
Negative control well median, by plate:	7.77315211624015
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-68
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-82.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.34941708254929
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Crespi CL, Miller VP, and Penman BW (1998) High throughput screening for inhibition of cytochrome P450 metabolism. Med Chem Res 8: 457-471.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2A2

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily a, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2A2, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2a2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2A2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.2 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4424
Neutral control median absolute deviation, by plate:	68.1996
Positive control well median response value, by plate:	825
Positive control well median absolute deviation, by plate:	22.239
Negative control well median, by plate:	106.645694062131
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-35
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.03
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

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Nominal number of tested concentrations:	8
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily a, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

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1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
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Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

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Assay Objectives:

NVS_ADME_rCYP2A2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.2 hour after chemical dosing in a 96-well plate.

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Negative control well median, by plate:	22.7617602427921
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-35
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.03
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.91806015509037
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2B1

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily b, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2B1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2B1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2b1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2B1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2B1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2B1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32325
Neutral control median absolute deviation, by plate:	613.055
Positive control well median response value, by plate:	1195
Positive control well median absolute deviation, by plate:	50.4084
Negative control well median, by plate:	108.788577895791
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-50
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.57849737099665
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2B1_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily b, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2B1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2B1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2b1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2B1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2B1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2B1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32325
Neutral control median absolute deviation, by plate:	613.055
Positive control well median response value, by plate:	1195
Positive control well median absolute deviation, by plate:	50.4084
Negative control well median, by plate:	8.08857273978889
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-50
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.57849737099665
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C6

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily C, polypeptide 6, variant 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C6, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c6v1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	45831
Neutral control median absolute deviation, by plate:	874.734
Positive control well median response value, by plate:	4349
Positive control well median absolute deviation, by plate:	124.538
Negative control well median, by plate:	100.76375539045
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-47.98
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.88050923509851
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

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Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C6_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily C, polypeptide 6, variant 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C6_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c6v1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C6, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	45831
Neutral control median absolute deviation, by plate:	874.734
Positive control well median response value, by plate:	4349
Positive control well median absolute deviation, by plate:	124.538
Negative control well median, by plate:	7.55057236945272
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-47.98
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.88050923509851
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C11

Assay Title: Novascreen rat unspecified cytochrome P450, subfamily 2, polypeptide 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C11 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C11, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c11. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C11, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C11 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C11 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.33 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	16307
Neutral control median absolute deviation, by plate:	135.658
Positive control well median response value, by plate:	1047.5
Positive control well median absolute deviation, by plate:	34.8411
Negative control well median, by plate:	108.111380145278
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-137
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-252.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.09105010163613
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

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MIE, Molecular Initiating Event

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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Date of Assay Document Creation:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C11_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, subfamily 2, polypeptide 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C11 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C11_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c11. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C11, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C11 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

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Assay Objectives:

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Assay Quality Statistics:

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Negative control well median, by plate:	21.0575646332891
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Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-137
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-252.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C12

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily c, polypeptide 12 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C12 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C12, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c12. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C12, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C12 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C12 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	14084.5
Neutral control median absolute deviation, by plate:	364.72
Positive control well median response value, by plate:	3801.5
Positive control well median absolute deviation, by plate:	65.9757
Negative control well median, by plate:	96.9737250740376
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-34.11
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.26
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.10637206437439
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C12_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily c, polypeptide 12 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C12 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C12_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c12. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C12, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C12 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C12 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	14084.5
Neutral control median absolute deviation, by plate:	364.72
Positive control well median response value, by plate:	3801.5
Positive control well median absolute deviation, by plate:	65.9757
Negative control well median, by plate:	25.7280536350304
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-34.11
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.26
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.10637206437439
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

4.1 Resources

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C13

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily c, polypeptide 13 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C13 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C13, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c13. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C13, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C13 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C13 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2809
Neutral control median absolute deviation, by plate:	62.2692
Positive control well median response value, by plate:	863
Positive control well median absolute deviation, by plate:	19.2738
Negative control well median, by plate:	99.5477671000565
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-30.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.92662603349891
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

StrÃ¶m A, Westin S, Eguchi H, Gustafsson JA, Mode A. Characterization of orphan nuclear receptor binding elements in sex-differentiated members of the CYP2C gene family expressed in rat liver. J Biol Chem. 1995 May 12;270(19):11276-81. PubMed PMID: 7744763.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Str m A Westin S Eguchi H Gustafsson JA Mode A Characterization of orphan nuclear receptor binding elements in sex differentiated members of the CYP2C gene family expressed in rat liver J Biol Chem 1995 May 12 270 19 11276 81 PubMed PMID 7744763 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Str m A Westin S Eguchi H Gustafsson JA Mode A Characterization of orphan nuclear receptor binding elements in sex differentiated members of the CYP2C gene family expressed in rat liver J Biol Chem 1995 May 12 270 19 11276 81 PubMed PMID 7744763 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2C13_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily c, polypeptide 13 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2C13 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2C13_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2c13. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2C13, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2C13 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2C13 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2809
Neutral control median absolute deviation, by plate:	62.2692
Positive control well median response value, by plate:	863
Positive control well median absolute deviation, by plate:	19.2738
Negative control well median, by plate:	27.4548311076198
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-30.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.92662603349891
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

StrÃ¶m A, Westin S, Eguchi H, Gustafsson JA, Mode A. Characterization of orphan nuclear receptor binding elements in sex-differentiated members of the CYP2C gene family expressed in rat liver. J Biol Chem. 1995 May 12;270(19):11276-81. PubMed PMID: 7744763.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Str m A Westin S Eguchi H Gustafsson JA Mode A Characterization of orphan nuclear receptor binding elements in sex differentiated members of the CYP2C gene family expressed in rat liver J Biol Chem 1995 May 12 270 19 11276 81 PubMed PMID 7744763 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Str m A Westin S Eguchi H Gustafsson JA Mode A Characterization of orphan nuclear receptor binding elements in sex differentiated members of the CYP2C gene family expressed in rat liver J Biol Chem 1995 May 12 270 19 11276 81 PubMed PMID 7744763 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2D1

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily d, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2D1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2D1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2d1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2D1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2D1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2D1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13100.5
Neutral control median absolute deviation, by plate:	97.1103
Positive control well median response value, by plate:	736.5
Positive control well median absolute deviation, by plate:	34.8411
Negative control well median, by plate:	101.920037192004
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-117
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-141.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.38012259194395
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2D1_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily d, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2D1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2D1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2d1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2D1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2D1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2D1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13100.5
Neutral control median absolute deviation, by plate:	97.1103
Positive control well median response value, by plate:	736.5
Positive control well median absolute deviation, by plate:	34.8411
Negative control well median, by plate:	38.1139983235541
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-117
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-141.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Nominal number of tested concentrations:	8
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Chemical Library Scope and Limitations:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2D2

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily d, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2D2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2D2, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2d2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2D2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2D2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2D2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17178.5
Neutral control median absolute deviation, by plate:	418.834
Positive control well median response value, by plate:	2877
Positive control well median absolute deviation, by plate:	63.7518
Negative control well median, by plate:	104.843630816171
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.12024963464081
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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NVS_ADME_rCYP2D2_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily d, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2D2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2D2_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2d2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2D2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2D2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Negative control well median, by plate:	12.7610045146727
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

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3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2E1

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily e, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2E1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2E1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2e1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2E1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2E1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2E1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	18882.5
Neutral control median absolute deviation, by plate:	193.479
Positive control well median response value, by plate:	4909
Positive control well median absolute deviation, by plate:	238.699
Negative control well median, by plate:	89.3261537332466
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-95
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-113.5
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.95842219064682
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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FBS, Fetal Bovine Serum

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NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP2E1_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 2, subfamily e, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP2E1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP2E1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp2e1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP2E1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP2E1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP2E1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	18882.5
Neutral control median absolute deviation, by plate:	193.479
Positive control well median response value, by plate:	4909
Positive control well median absolute deviation, by plate:	238.699
Negative control well median, by plate:	14.6135787978007
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-95
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-113.5
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.95842219064682
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP3A1

Assay Title: Novascreen rat unspecified cytochrome P450, family 3, subfamily a, polypeptide 23/polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP3A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP3A1, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp3a23/3a1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP3A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP3A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP3A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19176
Neutral control median absolute deviation, by plate:	151.966
Positive control well median response value, by plate:	1701
Positive control well median absolute deviation, by plate:	27.4281
Negative control well median, by plate:	103.496977920316
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-111
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-113.59
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.20626296963301
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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Assay Title: Novascreen rat unspecified cytochrome P450, family 3, subfamily a, polypeptide 23/polypeptide 1 assay

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1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP3A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP3A1_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp3a23/3a1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP3A1, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP3A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP3A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19176
Neutral control median absolute deviation, by plate:	151.966
Positive control well median response value, by plate:	1701
Positive control well median absolute deviation, by plate:	27.4281
Negative control well median, by plate:	7.69874563118372
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-111
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-113.59
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.20626296963301
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP3A2

Assay Title: Novascreen rat unspecified cytochrome P450, family 3, subfamily a, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP3A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP3A2, was analyzed in the positive fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp3a2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP3A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP3A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP3A2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32153
Neutral control median absolute deviation, by plate:	232.768
Positive control well median response value, by plate:	1845
Positive control well median absolute deviation, by plate:	29.652
Negative control well median, by plate:	105.098795869511
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-130
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-131.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.1453989585559
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ADME_rCYP3A2_Activator

Assay Title: Novascreen rat unspecified cytochrome P450, family 3, subfamily a, polypeptide 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ADME_rCYP3A2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ADME_rCYP3A2_Activator, was analyzed in the negative fitting direction relative to Acetonitrile as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Cyp3a2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "cyp" intended target family, where the subfamily is "xenobiotic metabolism". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ADME_rCYP3A2, is one of 1 assay component(s) measured or calculated from the NVS_ADME_rCYP3A2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ADME_rCYP3A2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32153
Neutral control median absolute deviation, by plate:	232.768
Positive control well median response value, by plate:	1845
Positive control well median absolute deviation, by plate:	29.652
Negative control well median, by plate:	65.8834553847222
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-130
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-131.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0089999999999999 µM
Standard maximum concentration tested:	19.9999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.1453989585559
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Stresser DM, Turner SD, Blanchard AP, Miller VP, Crespi CL. Cytochrome P450 fluorometric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. Drug Metab Dispos. 2002 Jul;30(7):845-52. PubMed PMID: 12065444.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAbl

Assay Title: Novascreen human unspecified ABL proto-oncogene 1, non-receptor tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAbl was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAbl, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ABL1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAbl, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAbl assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAbl is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.06
Neutral control median absolute deviation, by plate:	3.6472
Positive control well median response value, by plate:	0.18
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	98.5785358919688
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.63
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.31716417910447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Reddy EP, Smith MJ, Srinivasan A. Nucleotide sequence of Abelson murine leukemia virus genome: structural similarity of its transforming gene product to other onc gene products with tyrosine-specific kinase activity. Proc Natl Acad Sci U S A. 1983 Jun;80(12):3623-7. PubMed PMID: 6304726; PubMed Central PMCID: PMC394102.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAbl_Activator

Assay Title: Novascreen human unspecified ABL proto-oncogene 1, non-receptor tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAbl was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAbl_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ABL1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAbl, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAbl assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAbl is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.06
Neutral control median absolute deviation, by plate:	3.6472
Positive control well median response value, by plate:	0.18
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	92.5373134328358
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.63
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.31716417910447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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DMSO, Dimethyl Sulfoxide

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAChE

Assay Title: Novascreen human unspecified acetylcholinesterase (Yt blood group) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAChE was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAChE, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ACHE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "acetylcholinesterase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAChE, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAChE assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAChE is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.33 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.351
Neutral control median absolute deviation, by plate:	0.0044478
Positive control well median response value, by plate:	0.184
Positive control well median absolute deviation, by plate:	0.0014826
Negative control well median, by plate:	100.577293136626
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-165
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-195.38
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.32761634846971
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

ELLMAN GL, COURTNEY KD, ANDRES V Jr, FEATHER-STONE RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul;7:88-95. PubMed PMID: 13726518.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAChE_Activator

Assay Title: Novascreen human unspecified acetylcholinesterase (Yt blood group) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAChE was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAChE_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ACHE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "acetylcholinesterase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAChE, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAChE assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAChE is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.33 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.351
Neutral control median absolute deviation, by plate:	0.0044478
Positive control well median response value, by plate:	0.184
Positive control well median absolute deviation, by plate:	0.0014826
Negative control well median, by plate:	49.6997998665777
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-165
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-195.38
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.32761634846971
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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References:

ELLMAN GL, COURTNEY KD, ANDRES V Jr, FEATHER-STONE RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul;7:88-95. PubMed PMID: 13726518.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hACP1

Assay Title: Novascreen human unspecified acid phosphatase 1, soluble assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hACP1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hACP1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ACP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "acid phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hACP1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hACP1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hACP1 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.02
Neutral control median absolute deviation, by plate:	0.415128
Positive control well median response value, by plate:	4.56
Positive control well median absolute deviation, by plate:	0.830256
Negative control well median, by plate:	102.155887230514
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-78.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.40192503176619
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Assay Source Information:

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hACP1_Activator

Assay Title: Novascreen human unspecified acid phosphatase 1, soluble assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hACP1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hACP1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ACP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "acid phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hACP1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hACP1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hACP1 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.02
Neutral control median absolute deviation, by plate:	0.415128
Positive control well median response value, by plate:	4.56
Positive control well median absolute deviation, by plate:	0.830256
Negative control well median, by plate:	28.5464709993012
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-78.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.40192503176619
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAKT1

Assay Title: Novascreen human unspecified v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAKT1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAKT1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AKT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAKT1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAKT1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAKT1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	15.415
Neutral control median absolute deviation, by plate:	0.422541
Positive control well median response value, by plate:	0.14
Positive control well median absolute deviation, by plate:	0.051891
Negative control well median, by plate:	99.735516240874
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-56
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-56.69
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.94757399971782
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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NVS_ENZ_hAKT1_Activator

Assay Title: Novascreen human unspecified v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAKT1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAKT1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AKT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAKT1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAKT1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAKT1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	15.415
Neutral control median absolute deviation, by plate:	0.422541
Positive control well median response value, by plate:	0.14
Positive control well median absolute deviation, by plate:	0.051891
Negative control well median, by plate:	84.1301907968574
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-56
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-56.69
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.94757399971782
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAKT2

Assay Title: Novascreen human unspecified v-akt murine thymoma viral oncogene homolog 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAKT2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAKT2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AKT2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAKT2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAKT2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAKT2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	22.39
Neutral control median absolute deviation, by plate:	0.66717
Positive control well median response value, by plate:	0.37
Positive control well median absolute deviation, by plate:	0.088956
Negative control well median, by plate:	99.1421207658321
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-37
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-38.92
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.18905856005445
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAKT2_Activator

Assay Title: Novascreen human unspecified v-akt murine thymoma viral oncogene homolog 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAKT2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAKT2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AKT2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAKT2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAKT2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAKT2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	22.39
Neutral control median absolute deviation, by plate:	0.66717
Positive control well median response value, by plate:	0.37
Positive control well median absolute deviation, by plate:	0.088956
Negative control well median, by plate:	34.8958333333333
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-37
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-38.92
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.18905856005445
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAKT3

Assay Title: Novascreen human unspecified v-akt murine thymoma viral oncogene homolog 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAKT3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAKT3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AKT3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAKT3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAKT3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAKT3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.13
Neutral control median absolute deviation, by plate:	0.44478
Positive control well median response value, by plate:	0.09
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	100
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-76
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-78.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.90293782087849
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAKT3_Activator

Assay Title: Novascreen human unspecified v-akt murine thymoma viral oncogene homolog 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAKT3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAKT3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AKT3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAKT3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAKT3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAKT3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.13
Neutral control median absolute deviation, by plate:	0.44478
Positive control well median response value, by plate:	0.09
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	5.43354249857387
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-76
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-78.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.90293782087849
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAMPKa1

Assay Title: Novascreen human unspecified protein kinase, AMP-activated, alpha 1 catalytic subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAMPKa1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAMPKa1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKAA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAMPKa1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAMPKa1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAMPKa1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.07
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	0.28
Positive control well median absolute deviation, by plate:	0.088956
Negative control well median, by plate:	99.4281284969755
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-74
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-79.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.00671130764906
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAMPKa1_Activator

Assay Title: Novascreen human unspecified protein kinase, AMP-activated, alpha 1 catalytic subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAMPKa1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAMPKa1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKAA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAMPKa1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAMPKa1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAMPKa1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.07
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	0.28
Positive control well median absolute deviation, by plate:	0.088956
Negative control well median, by plate:	22.1263638670388
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-74
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-79.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAurA

Assay Title: Novascreen human unspecified aurora kinase A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAurA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAurA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AURKA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAurA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAurA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAurA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.48
Neutral control median absolute deviation, by plate:	1.58638
Positive control well median response value, by plate:	0.56
Positive control well median absolute deviation, by plate:	0.281694
Negative control well median, by plate:	100.645161290323
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.93486952202312
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hAurA_Activator

Assay Title: Novascreen human unspecified aurora kinase A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hAurA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hAurA_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene AURKA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hAurA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hAurA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hAurA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.48
Neutral control median absolute deviation, by plate:	1.58638
Positive control well median response value, by plate:	0.56
Positive control well median absolute deviation, by plate:	0.281694
Negative control well median, by plate:	20.0713544266685
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.93486952202312
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hBACE

Assay Title: Novascreen human unspecified beta-site APP-cleaving enzyme 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hBACE was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hBACE, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene BACE1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "aspartate protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hBACE, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hBACE assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hBACE is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9270
Neutral control median absolute deviation, by plate:	117.125
Positive control well median response value, by plate:	2478
Positive control well median absolute deviation, by plate:	17.7912
Negative control well median, by plate:	423.847355868081
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-46
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.80784656796769
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature. 1999 Dec 2;402(6761):537-40. PubMed PMID: 10591214.|Citron M. Human beta-secretase and Alzheimer's disease. Expert Opin Ther Targets. 2001 Jun;5(3):341-348. PubMed PMID: 12540269.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID

10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hBACE_Activator

Assay Title: Novascreen human unspecified beta-site APP-cleaving enzyme 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hBACE was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hBACE_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene BACE1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "aspartate protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hBACE, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hBACE assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hBACE is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9270
Neutral control median absolute deviation, by plate:	117.125
Positive control well median response value, by plate:	2478
Positive control well median absolute deviation, by plate:	17.7912
Negative control well median, by plate:	83.7718288551978
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-46
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.80784656796769
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature. 1999 Dec 2;402(6761):537-40. PubMed PMID: 10591214.|Citron M. Human beta-secretase and Alzheimer's disease. Expert Opin Ther Targets. 2001 Jun;5(3):341-348. PubMed PMID: 12540269.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor

protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sinha S Anderson JP Barbour R Basi GS Caccavello R Davis D Doan M Dovey HF Frigon N Hong J Jacobson Croak K Jewett N Keim P Knops J Lieberburg I Power M Tan H Tatsuno G Tung J Schenk D Seubert P Suomensaari SM Wang S Walker D Zhao J McConlogue L John V Purification and cloning of amyloid precursor protein beta secretase from human brain Nature 1999 Dec 2 402 6761 537 40 PubMed PMID 10591214 Citron M Human beta secretase and Alzheimer s disease Expert Opin Ther Targets 2001 Jun 5 3 341 348 PubMed PMID 12540269 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hBTK

Assay Title: Novascreen human unspecified Bruton agammaglobulinemia tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hBTK was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hBTK, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene BTK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hBTK, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hBTK assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hBTK is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.53
Neutral control median absolute deviation, by plate:	3.72133
Positive control well median response value, by plate:	0.115
Positive control well median absolute deviation, by plate:	0.051891
Negative control well median, by plate:	99.0117214433463
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.54
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-6.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.15

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.66446104343829
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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NVS_ENZ_hBTK_Activator

Assay Title: Novascreen human unspecified Bruton agammaglobulinemia tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hBTK was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hBTK_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene BTK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hBTK, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hBTK assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Negative control well median, by plate:	46.655941162951
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.54
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-7
SSMD (median across all plates, using negative control wells):	unspecified
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Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
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CV (median across all plates):	0.15

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unspecified

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All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.66446104343829
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP1

Assay Title: Novascreen human unspecified caspase 1, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP1 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	76537
Neutral control median absolute deviation, by plate:	364.72
Positive control well median response value, by plate:	6274
Positive control well median absolute deviation, by plate:	131.951
Negative control well median, by plate:	93.3978814297001
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-155
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-175.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.51149587450342
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP1_Activator

Assay Title: Novascreen human unspecified caspase 1, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP1 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	76537
Neutral control median absolute deviation, by plate:	364.72
Positive control well median response value, by plate:	6274
Positive control well median absolute deviation, by plate:	131.951
Negative control well median, by plate:	21.7649592255189
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-155
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-175.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.51149587450342
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP2

Assay Title: Novascreen human unspecified caspase 2, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP2 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17700
Neutral control median absolute deviation, by plate:	133.434
Positive control well median response value, by plate:	1962
Positive control well median absolute deviation, by plate:	31.1346
Negative control well median, by plate:	102.128065291179
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-83
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-98.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.49539215266146
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP2_Activator

Assay Title: Novascreen human unspecified caspase 2, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP2 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17700
Neutral control median absolute deviation, by plate:	133.434
Positive control well median response value, by plate:	1962
Positive control well median absolute deviation, by plate:	31.1346
Negative control well median, by plate:	33.356070941337
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-83
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-98.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.49539215266146
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Talanian RV, Quinlan C, Trautz S, Hackett MC, Mankovich JA, Banach D, Ghayur T, Brady KD, Wong WW. Substrate specificities of caspase family proteases. J Biol Chem. 1997 Apr 11;272(15):9677-82. PubMed PMID: 9092497.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP3

Assay Title: Novascreen human unspecified caspase 3, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP3 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5286
Neutral control median absolute deviation, by plate:	5.9304
Positive control well median response value, by plate:	2290
Positive control well median absolute deviation, by plate:	16.3086
Negative control well median, by plate:	103.299832495812
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-149
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1018.31
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.42
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.2727405822286
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Talanian RV, Quinlan C, Trautz S, Hackett MC, Mankovich JA, Banach D, Ghayur T, Brady KD, Wong WW. Substrate specificities of caspase family proteases. J Biol Chem. 1997 Apr 11;272(15):9677-82. PubMed PMID: 9092497.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP3_Activator

Assay Title: Novascreen human unspecified caspase 3, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP3 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5286
Neutral control median absolute deviation, by plate:	5.9304
Positive control well median response value, by plate:	2290
Positive control well median absolute deviation, by plate:	16.3086
Negative control well median, by plate:	31.0089020771513
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-149
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1018.31
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.42
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
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Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.2727405822286
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP4

Assay Title: Novascreen human unspecified caspase 4, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP4 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10497
Neutral control median absolute deviation, by plate:	54.8562
Positive control well median response value, by plate:	4171
Positive control well median absolute deviation, by plate:	71.1648
Negative control well median, by plate:	94.0699902334999
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-117.63
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.4
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.2700890352387
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Talanian RV, Quinlan C, Trautz S, Hackett MC, Mankovich JA, Banach D, Ghayur T, Brady KD, Wong WW. Substrate specificities of caspase family proteases. J Biol Chem. 1997 Apr 11;272(15):9677-82. PubMed PMID: 9092497.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP4_Activator

Assay Title: Novascreen human unspecified caspase 4, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP4_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP4 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10497
Neutral control median absolute deviation, by plate:	54.8562
Positive control well median response value, by plate:	4171
Positive control well median absolute deviation, by plate:	71.1648
Negative control well median, by plate:	720.414201183432
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-117.63
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.4
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.2700890352387
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP5

Assay Title: Novascreen human unspecified caspase 5, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP5, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP5, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP5 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	20177
Neutral control median absolute deviation, by plate:	105.265
Positive control well median response value, by plate:	3005
Positive control well median absolute deviation, by plate:	14.826
Negative control well median, by plate:	112.711515712596
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-62
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-173.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.3235688754468
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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NVS_ENZ_hCASP5_Activator

Assay Title: Novascreen human unspecified caspase 5, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP5_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP5, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

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Negative control well median, by plate:	76.9809825673534
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-62
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-173.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.3235688754468
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Talanian RV, Quinlan C, Trautz S, Hackett MC, Mankovich JA, Banach D, Ghayur T, Brady KD, Wong WW. Substrate specificities of caspase family proteases. J Biol Chem. 1997 Apr 11;272(15):9677-82. PubMed PMID: 9092497.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP8

Assay Title: Novascreen human unspecified caspase 8, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP8 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP8, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP8, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP8 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP8 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30529
Neutral control median absolute deviation, by plate:	235.733
Positive control well median response value, by plate:	3168.5
Positive control well median absolute deviation, by plate:	48.9258
Negative control well median, by plate:	98.0517437947427
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-109
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-118.8
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.45426433410861
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP8_Activator

Assay Title: Novascreen human unspecified caspase 8, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP8 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP8_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP8. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP8, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP8 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP8 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30529
Neutral control median absolute deviation, by plate:	235.733
Positive control well median response value, by plate:	3168.5
Positive control well median absolute deviation, by plate:	48.9258
Negative control well median, by plate:	27.7328385899815
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-109
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-118.8
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.45426433410861
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP10

Assay Title: Novascreen human unspecified caspase 10, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP10 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP10, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP10, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP10 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP10 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	14487
Neutral control median absolute deviation, by plate:	93.4038
Positive control well median response value, by plate:	2457
Positive control well median absolute deviation, by plate:	60.7866
Negative control well median, by plate:	98.006127441027
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-90
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-129.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCASP10_Activator

Assay Title: Novascreen human unspecified caspase 10, apoptosis-related cysteine peptidase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCASP10 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCASP10_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CASP10. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "cysteine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCASP10, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCASP10 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCASP10 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	14487
Neutral control median absolute deviation, by plate:	93.4038
Positive control well median response value, by plate:	2457
Positive control well median absolute deviation, by plate:	60.7866
Negative control well median, by plate:	21.2669116418908
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-90
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-129.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.74738543778287
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Talanian RV, Quinlan C, Trautz S, Hackett MC, Mankovich JA, Banach D, Ghayur T, Brady KD, Wong WW. Substrate specificities of caspase family proteases. J Biol Chem. 1997 Apr 11;272(15):9677-82. PubMed PMID: 9092497.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCDK2

Assay Title: Novascreen human unspecified cyclin-dependent kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCDK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCDK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CDK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCDK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCDK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCDK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.125
Neutral control median absolute deviation, by plate:	0.259455
Positive control well median response value, by plate:	1.48
Positive control well median absolute deviation, by plate:	0.318759
Negative control well median, by plate:	99.0417174691256
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-140.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.08502939090467
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCDK2_Activator

Assay Title: Novascreen human unspecified cyclin-dependent kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCDK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCDK2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CDK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCDK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCDK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCDK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.125
Neutral control median absolute deviation, by plate:	0.259455
Positive control well median response value, by plate:	1.48
Positive control well median absolute deviation, by plate:	0.318759
Negative control well median, by plate:	21.0264266564535
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-140.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.08502939090467
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCDK6

Assay Title: Novascreen human unspecified cyclin-dependent kinase 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCDK6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCDK6, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CDK6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCDK6, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCDK6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCDK6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30.68
Neutral control median absolute deviation, by plate:	1.37882
Positive control well median response value, by plate:	3.02
Positive control well median absolute deviation, by plate:	0.103782
Negative control well median, by plate:	99.9484536082474
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-20.06
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.66828865979381
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.0097319587629

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCDK6_Activator

Assay Title: Novascreen human unspecified cyclin-dependent kinase 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCDK6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCDK6_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CDK6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCDK6, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCDK6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCDK6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30.68
Neutral control median absolute deviation, by plate:	1.37882
Positive control well median response value, by plate:	3.02
Positive control well median absolute deviation, by plate:	0.103782
Negative control well median, by plate:	7.7835051546392
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-20.06
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.66828865979381
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.0097319587629

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCHK1

Assay Title: Novascreen human unspecified checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCHK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCHK1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CHEK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCHK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCHK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCHK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	44.03
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	0.92
Positive control well median absolute deviation, by plate:	0.415128
Negative control well median, by plate:	100
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-64
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-80.77
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5.5
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	22.3606797749979 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.02728944932902
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCHK1_Activator

Assay Title: Novascreen human unspecified checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCHK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCHK1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CHEK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCHK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCHK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCHK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	44.03
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	0.92
Positive control well median absolute deviation, by plate:	0.415128
Negative control well median, by plate:	4.87043035631652
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-64
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-80.77
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5.5
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	22.3606797749979 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.02728944932902
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCK1a

Assay Title: Novascreen human unspecified casein kinase 1, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCK1a was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCK1a, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSNK1A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCK1a, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCK1a assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCK1a is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.55
Neutral control median absolute deviation, by plate:	0.385476
Positive control well median response value, by plate:	0.68
Positive control well median absolute deviation, by plate:	0.103782
Negative control well median, by plate:	97.8794304756134
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-85
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-87.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.20076946379885
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCK1a_Activator

Assay Title: Novascreen human unspecified casein kinase 1, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCK1a was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCK1a_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSNK1A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCK1a, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCK1a assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCK1a is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.55
Neutral control median absolute deviation, by plate:	0.385476
Positive control well median response value, by plate:	0.68
Positive control well median absolute deviation, by plate:	0.103782
Negative control well median, by plate:	13.6928203574674
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-85
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-87.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.20076946379885
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCK1D

Assay Title: Novascreen human unspecified casein kinase 1, delta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCK1D was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCK1D, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSNK1D. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCK1D, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCK1D assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCK1D is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	20.79
Neutral control median absolute deviation, by plate:	0.578214
Positive control well median response value, by plate:	0.47
Positive control well median absolute deviation, by plate:	0.007413
Negative control well median, by plate:	99.5372443648306
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-45
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.23417406624382
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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NVS_ENZ_hCK1D_Activator

Assay Title: Novascreen human unspecified casein kinase 1, delta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCK1D was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCK1D_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSNK1D. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCK1D, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCK1D assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCK1D is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	20.79
Neutral control median absolute deviation, by plate:	0.578214
Positive control well median response value, by plate:	0.47
Positive control well median absolute deviation, by plate:	0.007413
Negative control well median, by plate:	101.854754440961
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-45
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.23417406624382
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCK2a2b2

Assay Title: Novascreen human unspecified casein kinase 2, alpha 1 polypeptide assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCK2a2b2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCK2a2b2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSNK2A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCK2a2b2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCK2a2b2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCK2a2b2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	44.4
Neutral control median absolute deviation, by plate:	0.103782
Positive control well median response value, by plate:	6.04
Positive control well median absolute deviation, by plate:	0.474432
Negative control well median, by plate:	112.210027438264
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-79
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-369.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.32985283112996
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCK2a2b2_Activator

Assay Title: Novascreen human unspecified casein kinase 2, alpha 1 polypeptide assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCK2a2b2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCK2a2b2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSNK2A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCK2a2b2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCK2a2b2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCK2a2b2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	44.4
Neutral control median absolute deviation, by plate:	0.103782
Positive control well median response value, by plate:	6.04
Positive control well median absolute deviation, by plate:	0.474432
Negative control well median, by plate:	13.1329508605637
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-79
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-369.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.32985283112996
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCSF1R

Assay Title: Novascreen human unspecified colony stimulating factor 1 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCSF1R was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCSF1R, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSF1R. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCSF1R, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCSF1R assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCSF1R is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.005
Neutral control median absolute deviation, by plate:	0.578214
Positive control well median response value, by plate:	0.295
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	99.9050817757009
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-53
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-54.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.96025334912129
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hCSF1R_Activator

Assay Title: Novascreen human unspecified colony stimulating factor 1 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hCSF1R was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hCSF1R_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CSF1R. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hCSF1R, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hCSF1R assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hCSF1R is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.005
Neutral control median absolute deviation, by plate:	0.578214
Positive control well median response value, by plate:	0.295
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	40.5050505050505
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-53
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-54.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.96025334912129
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hDUSP3

Assay Title: Novascreen human unspecified dual specificity phosphatase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hDUSP3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hDUSP3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene DUSP3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "dual-specific phosphatase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hDUSP3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hDUSP3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hDUSP3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	56506.5
Neutral control median absolute deviation, by plate:	321.724
Positive control well median response value, by plate:	4586.5
Positive control well median absolute deviation, by plate:	199.039
Negative control well median, by plate:	120.900237160297
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-51
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-72.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.24402699038699
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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Kulas DT, Zhang WR, Goldstein BJ, Furlanetto RW, Mooney RA. Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR. J Biol Chem. 1995 Feb 10;270(6):2435-8. PubMed PMID: 7852302.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR J Biol Chem 1995 Feb 10 270 6 2435 8 PubMed PMID 7852302 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR J Biol Chem 1995 Feb 10 270 6 2435 8 PubMed PMID 7852302 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR J Biol

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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<u>Contact Information:</u>

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hDUSP3_Activator

Assay Title: Novascreen human unspecified dual specificity phosphatase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hDUSP3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hDUSP3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene DUSP3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "dual-specific phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hDUSP3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hDUSP3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hDUSP3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	56506.5
Neutral control median absolute deviation, by plate:	321.724
Positive control well median response value, by plate:	4586.5
Positive control well median absolute deviation, by plate:	199.039
Negative control well median, by plate:	56.1057108140948
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-51
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-72.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.24402699038699
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Kulas DT, Zhang WR, Goldstein BJ, Furlanetto RW, Mooney RA. Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR. J Biol Chem. 1995 Feb 10;270(6):2435-8. PubMed PMID: 7852302.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR J Biol Chem 1995 Feb 10 270 6 2435 8 PubMed PMID 7852302 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR J Biol Chem 1995 Feb 10 270 6 2435 8 PubMed PMID 7852302 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin

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Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Kulas DT Zhang WR Goldstein BJ Furlanetto RW Mooney RA Insulin receptor signaling is augmented by antisense inhibition of the protein tyrosine phosphatase LAR J Biol Chem 1995 Feb 10 270 6 2435 8 PubMed PMID 7852302 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hDYRK1a

Assay Title: Novascreen human unspecified dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hDYRK1a was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hDYRK1a, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene DYRK1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "dual-specific kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hDYRK1a, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hDYRK1a assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hDYRK1a is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.72
Neutral control median absolute deviation, by plate:	0.237216
Positive control well median response value, by plate:	1.56
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	98.7997133643855
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-94
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-114.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.4355822285919
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6134933715514

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hDYRK1a_Activator

Assay Title: Novascreen human unspecified dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hDYRK1a was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hDYRK1a_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene DYRK1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "dual-specific kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hDYRK1a, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hDYRK1a assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hDYRK1a is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.72
Neutral control median absolute deviation, by plate:	0.237216
Positive control well median response value, by plate:	1.56
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	12.7194553923325
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-94
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-114.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.4355822285919
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6134933715514

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEGFR

Assay Title: Novascreen human unspecified epidermal growth factor receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEGFR was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEGFR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EGFR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEGFR, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEGFR assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEGFR is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.97
Neutral control median absolute deviation, by plate:	0.578214
Positive control well median response value, by plate:	1.5
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	102.201665124884
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-60.86
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.60329227996397
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEGFR_Activator

Assay Title: Novascreen human unspecified epidermal growth factor receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEGFR was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEGFR_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EGFR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEGFR, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEGFR assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEGFR is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.97
Neutral control median absolute deviation, by plate:	0.578214
Positive control well median response value, by plate:	1.5
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	13.7278462000601
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-60.86
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.60329227996397
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hElastase

Assay Title: Novascreen human unspecified elastase, neutrophil expressed assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hElastase was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hElastase, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ELANE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hElastase, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hElastase assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Spectrophotometry technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hElastase is a biochemical, single-readout assay that uses extracted gene-proteins from Human neutrophils in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.42
Neutral control median absolute deviation, by plate:	0.0014826
Positive control well median response value, by plate:	0.068
Positive control well median absolute deviation, by plate:	0.0014826
Negative control well median, by plate:	108.641975308642
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-91
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-132.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.98040145867003
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

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Safayhi H, Rall B, Sailer ER, Ammon HP. Inhibition by boswellic acids of human leukocyte elastase. J Pharmacol Exp Ther. 1997 Apr;281(1):460-3. PubMed PMID: 9103531.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Safayhi H Rall B Sailer ER Ammon HP Inhibition by boswellic acids of human leukocyte elastase J Pharmacol Exp Ther 1997 Apr 281 1 460 3 PubMed PMID 9103531 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Safayhi H Rall B Sailer ER Ammon HP Inhibition by boswellic acids of human leukocyte elastase J Pharmacol Exp Ther 1997 Apr 281 1 460 3 PubMed PMID 9103531 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hElastase_Activator

Assay Title: Novascreen human unspecified elastase, neutrophil expressed assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hElastase was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hElastase_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ELANE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "serine protease". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hElastase, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hElastase assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Spectrophotometry technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hElastase is a biochemical, single-readout assay that uses extracted gene-proteins from Human neutrophils in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.42
Neutral control median absolute deviation, by plate:	0.0014826
Positive control well median response value, by plate:	0.068
Positive control well median absolute deviation, by plate:	0.0014826
Negative control well median, by plate:	27.3390036452005
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-91
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-132.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.98040145867003
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Safayhi H, Rall B, Sailer ER, Ammon HP. Inhibition by boswellic acids of human leukocyte elastase. J Pharmacol Exp Ther. 1997 Apr;281(1):460-3. PubMed PMID: 9103531.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Safayhi H Rall B Sailer ER Ammon HP Inhibition by boswellic acids of human leukocyte elastase J Pharmacol Exp Ther 1997 Apr 281 1 460 3 PubMed PMID 9103531 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Safayhi H Rall B Sailer ER Ammon HP Inhibition by boswellic acids of human leukocyte elastase J Pharmacol Exp Ther 1997 Apr 281 1 460 3 PubMed PMID 9103531 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphA1

Assay Title: Novascreen human unspecified EPH receptor A1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphA1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphA1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphA1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphA1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphA1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30.295
Neutral control median absolute deviation, by plate:	0.281694
Positive control well median response value, by plate:	16.865
Positive control well median absolute deviation, by plate:	23.8773
Negative control well median, by plate:	100.979895252576
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-80
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-124.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.156683368353
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphA1_Activator

Assay Title: Novascreen human unspecified EPH receptor A1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphA1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphA1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHA1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphA1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphA1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphA1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30.295
Neutral control median absolute deviation, by plate:	0.281694
Positive control well median response value, by plate:	16.865
Positive control well median absolute deviation, by plate:	23.8773
Negative control well median, by plate:	43.6513268390998
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-80
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-124.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphA2

Assay Title: Novascreen human unspecified EPH receptor A2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphA2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphA2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHA2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphA2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphA2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphA2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.305
Neutral control median absolute deviation, by plate:	0.452193
Positive control well median response value, by plate:	0.99
Positive control well median absolute deviation, by plate:	0.155673
Negative control well median, by plate:	112.087912087912
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-46.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.90076923076923
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphA2_Activator

Assay Title: Novascreen human unspecified EPH receptor A2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphA2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphA2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHA2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphA2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphA2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphA2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.305
Neutral control median absolute deviation, by plate:	0.452193
Positive control well median response value, by plate:	0.99
Positive control well median absolute deviation, by plate:	0.155673
Negative control well median, by plate:	161.777488048675
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-46.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.90076923076923
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphB1

Assay Title: Novascreen human unspecified EPH receptor B1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphB1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphB1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphB1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphB1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphB1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.06
Neutral control median absolute deviation, by plate:	0.489258
Positive control well median response value, by plate:	0.6
Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	100
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-67
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-70.43
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.68328221763493
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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4. Assay Documentation

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphB1_Activator

Assay Title: Novascreen human unspecified EPH receptor B1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphB1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphB1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphB1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphB1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphB1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.06
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Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	6.78265998230612
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-67
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-70.43
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Standard minimum concentration tested:	0.0199999999999999 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.68328221763493
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphB2

Assay Title: Novascreen human unspecified EPH receptor B2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphB2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphB2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHB2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphB2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphB2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphB2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17.325
Neutral control median absolute deviation, by plate:	0.22239
Positive control well median response value, by plate:	0.525
Positive control well median absolute deviation, by plate:	0.096369
Negative control well median, by plate:	99.3033762280949
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-90.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.67594036697248
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hEphB2_Activator

Assay Title: Novascreen human unspecified EPH receptor B2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hEphB2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hEphB2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene EPHB2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hEphB2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hEphB2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hEphB2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17.325
Neutral control median absolute deviation, by plate:	0.22239
Positive control well median response value, by plate:	0.525
Positive control well median absolute deviation, by plate:	0.096369
Negative control well median, by plate:	168.545216251638
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-90.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.67594036697248
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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References:

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hES

Assay Title: Novascreen human unspecified butyrylcholinesterase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hES was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hES, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene BCHE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "pseudocholinesterase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hES, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hES assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hES is a biochemical, single-readout assay that uses extracted gene-proteins from Human plasma in a cell-free assay.Measurements were taken 0.25 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.932
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	0.23
Positive control well median absolute deviation, by plate:	0.0029652
Negative control well median, by plate:	105.871670702179
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-208
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-229.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.614733428029295
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hES was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hES_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene BCHE. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "pseudocholinesterase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hES, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hES assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hES is a biochemical, single-readout assay that uses extracted gene-proteins from Human plasma in a cell-free assay.Measurements were taken 0.25 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.932
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	0.23
Positive control well median absolute deviation, by plate:	0.0029652
Negative control well median, by plate:	36.076512455516
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.98
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-208
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-229.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.614733428029295
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

ELLMAN GL, COURTNEY KD, ANDRES V Jr, FEATHER-STONE RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul;7:88-95. PubMed PMID: 13726518.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hFGFR1

Assay Title: Novascreen human unspecified fibroblast growth factor receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hFGFR1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hFGFR1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FGFR1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hFGFR1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hFGFR1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hFGFR1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	45.04
Neutral control median absolute deviation, by plate:	0.681996
Positive control well median response value, by plate:	0.3
Positive control well median absolute deviation, by plate:	0.07413
Negative control well median, by plate:	105.082495732979
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-56
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.13
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.42584033584568
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hFGFR1_Activator

Assay Title: Novascreen human unspecified fibroblast growth factor receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hFGFR1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hFGFR1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FGFR1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hFGFR1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hFGFR1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hFGFR1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	45.04
Neutral control median absolute deviation, by plate:	0.681996
Positive control well median response value, by plate:	0.3
Positive control well median absolute deviation, by plate:	0.07413
Negative control well median, by plate:	14.2664872139973
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-56
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.13
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.42584033584568
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hFGFR3

Assay Title: Novascreen human unspecified fibroblast growth factor receptor 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hFGFR3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hFGFR3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FGFR3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hFGFR3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hFGFR3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hFGFR3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	40.54
Neutral control median absolute deviation, by plate:	0.355824
Positive control well median response value, by plate:	1.13
Positive control well median absolute deviation, by plate:	0.415128
Negative control well median, by plate:	98.7037987679672
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-72
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-110.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.67439425051336
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hFGFR3_Activator

Assay Title: Novascreen human unspecified fibroblast growth factor receptor 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hFGFR3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hFGFR3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FGFR3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hFGFR3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hFGFR3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hFGFR3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	40.54
Neutral control median absolute deviation, by plate:	0.355824
Positive control well median response value, by plate:	1.13
Positive control well median absolute deviation, by plate:	0.415128
Negative control well median, by plate:	14.0913757700205
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-72
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-110.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.67439425051336
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hFyn

Assay Title: Novascreen human unspecified FYN proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hFyn was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hFyn, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FYN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hFyn, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hFyn assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hFyn is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	23.09
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	0.32
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	99.304520615996
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.24
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.17945516251246
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hFyn_Activator

Assay Title: Novascreen human unspecified FYN proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hFyn was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hFyn_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FYN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hFyn, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hFyn assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

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Assay Objectives:

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Assay Quality Statistics:

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Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	111.565304087737
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.24
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hGSK3b

Assay Title: Novascreen human unspecified glycogen synthase kinase 3 beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hGSK3b was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hGSK3b, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene GSK3B. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hGSK3b, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hGSK3b assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hGSK3b is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.62
Neutral control median absolute deviation, by plate:	0.244629
Positive control well median response value, by plate:	1.69
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	99.3991144845035
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-97
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-125.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.757541223309627
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Date of Assay Document Creation:

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NVS_ENZ_hGSK3b_Activator

Assay Title: Novascreen human unspecified glycogen synthase kinase 3 beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hGSK3b was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hGSK3b_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene GSK3B. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hGSK3b, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hGSK3b assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hGSK3b is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.62
Neutral control median absolute deviation, by plate:	0.244629
Positive control well median response value, by plate:	1.69
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	25.3872790231456
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-97
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-125.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.757541223309627
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hHDAC3

Assay Title: Novascreen human unspecified histone deacetylase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hHDAC3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hHDAC3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene HDAC3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "histone deacetylase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hHDAC3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hHDAC3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hHDAC3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.09
Neutral control median absolute deviation, by plate:	0.118608
Positive control well median response value, by plate:	0.74
Positive control well median absolute deviation, by plate:	0.177912
Negative control well median, by plate:	99.6713293408368
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-136
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-185.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.15231067344957
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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NVS_ENZ_hHDAC3_Activator

Assay Title: Novascreen human unspecified histone deacetylase 3 assay

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1.1 Overview

Assay Summary:

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1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hHDAC3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hHDAC3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

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Caveats:

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Assay Objectives:

NVS_ENZ_hHDAC3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

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Positive control well median absolute deviation, by plate:	0.177912
Negative control well median, by plate:	50.9022556390977
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-136
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-185.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.15231067344957
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hHDAC6

Assay Title: Novascreen human unspecified histone deacetylase 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hHDAC6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hHDAC6, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene HDAC6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "histone deacetylase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hHDAC6, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hHDAC6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hHDAC6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.995
Neutral control median absolute deviation, by plate:	1.19349
Positive control well median response value, by plate:	0.655
Positive control well median absolute deviation, by plate:	0.103782
Negative control well median, by plate:	104.845528455285
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36.19
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.44671543525969
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6802926115581

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hHDAC6_Activator

Assay Title: Novascreen human unspecified histone deacetylase 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hHDAC6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hHDAC6_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene HDAC6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "histone deacetylase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hHDAC6, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hHDAC6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hHDAC6 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.995
Neutral control median absolute deviation, by plate:	1.19349
Positive control well median response value, by plate:	0.655
Positive control well median absolute deviation, by plate:	0.103782
Negative control well median, by plate:	101.097293343087
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36.19
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.44671543525969
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6802926115581

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hIGF1R

Assay Title: Novascreen human unspecified insulin-like growth factor 1 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hIGF1R was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hIGF1R, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene IGF1R. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hIGF1R, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hIGF1R assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hIGF1R is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.12
Neutral control median absolute deviation, by plate:	0.800604
Positive control well median response value, by plate:	1.09
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	97.7989292088043
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-39
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-41.99
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.9954691864389
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hIGF1R_Activator

Assay Title: Novascreen human unspecified insulin-like growth factor 1 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hIGF1R was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hIGF1R_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene IGF1R. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hIGF1R, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hIGF1R assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hIGF1R is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.12
Neutral control median absolute deviation, by plate:	0.800604
Positive control well median response value, by plate:	1.09
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	124.742571344513
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-39
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-41.99
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.9954691864389
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hIKKa

Assay Title: Novascreen human unspecified conserved helix-loop-helix ubiquitous kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hIKKa was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hIKKa, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CHUK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hIKKa, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hIKKa assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hIKKa is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	25.02
Neutral control median absolute deviation, by plate:	0.415128
Positive control well median response value, by plate:	0.615
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	98.4486459504724
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-77.66
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.43197151836343
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

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NVS_ENZ_hIKKa_Activator

Assay Title: Novascreen human unspecified conserved helix-loop-helix ubiquitous kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hIKKa was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hIKKa_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CHUK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hIKKa, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hIKKa assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Assay Objectives:

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Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	0.415128
Positive control well median response value, by plate:	0.615
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	12.6260412099956
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-77.66
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hInsR

Assay Title: Novascreen human unspecified insulin receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hInsR was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hInsR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene INSR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hInsR, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hInsR assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hInsR is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.72
Neutral control median absolute deviation, by plate:	1.23056
Positive control well median response value, by plate:	0.62
Positive control well median absolute deviation, by plate:	0.207564
Negative control well median, by plate:	98.3685348411774
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.93496296296295
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hInsR_Activator

Assay Title: Novascreen human unspecified insulin receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hInsR was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hInsR_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene INSR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hInsR, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hInsR assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hInsR is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.72
Neutral control median absolute deviation, by plate:	1.23056
Positive control well median response value, by plate:	0.62
Positive control well median absolute deviation, by plate:	0.207564
Negative control well median, by plate:	212.664277180406
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.93496296296295
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hIRAK4

Assay Title: Novascreen human unspecified interleukin-1 receptor-associated kinase 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hIRAK4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hIRAK4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene IRAK4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hIRAK4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hIRAK4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hIRAK4 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.055
Neutral control median absolute deviation, by plate:	6.89409
Positive control well median response value, by plate:	0.02
Positive control well median absolute deviation, by plate:	0.467019
Negative control well median, by plate:	97.7272727272727
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.69

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	18.0677351097179
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	108.406410658307

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hIRAK4_Activator

Assay Title: Novascreen human unspecified interleukin-1 receptor-associated kinase 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hIRAK4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hIRAK4_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene IRAK4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hIRAK4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hIRAK4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hIRAK4 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10.055
Neutral control median absolute deviation, by plate:	6.89409
Positive control well median response value, by plate:	0.02
Positive control well median absolute deviation, by plate:	0.467019
Negative control well median, by plate:	295.611285266458
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.69

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	108.406410658307

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hJak2

Assay Title: Novascreen human unspecified Janus kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hJak2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hJak2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene JAK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hJak2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hJak2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hJak2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.77
Neutral control median absolute deviation, by plate:	0.548562
Positive control well median response value, by plate:	0.24
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	99.8284898674703
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-51
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-51.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.33205237624578
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hJak2_Activator

Assay Title: Novascreen human unspecified Janus kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hJak2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hJak2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene JAK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hJak2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hJak2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hJak2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.77
Neutral control median absolute deviation, by plate:	0.548562
Positive control well median response value, by plate:	0.24
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	12.2326883910387
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-51
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-51.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.33205237624578
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hJNK2

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hJNK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hJNK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hJNK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hJNK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hJNK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32.67
Neutral control median absolute deviation, by plate:	0.207564
Positive control well median response value, by plate:	4.49
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	99.1433466590518
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-83
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-135.77
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.16830828764207
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

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SSMD, Strictly Standardized Mean Difference

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hJNK2_Activator

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hJNK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hJNK2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hJNK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hJNK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hJNK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32.67
Neutral control median absolute deviation, by plate:	0.207564
Positive control well median response value, by plate:	4.49
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	6.48248465149872
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-83
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-135.77
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.16830828764207
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hLck

Assay Title: Novascreen human unspecified LCK proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hLck was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hLck, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene LCK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hLck, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hLck assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hLck is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.345
Neutral control median absolute deviation, by plate:	3.55083
Positive control well median response value, by plate:	0.21
Positive control well median absolute deviation, by plate:	0.140847
Negative control well median, by plate:	99.1487385853583
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.7
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-10
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-10.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.95048754062838
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hLck_Activator

Assay Title: Novascreen human unspecified LCK proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hLck was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hLck_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene LCK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hLck, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hLck assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hLck is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.345
Neutral control median absolute deviation, by plate:	3.55083
Positive control well median response value, by plate:	0.21
Positive control well median absolute deviation, by plate:	0.140847
Negative control well median, by plate:	49.1564773254914
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.7
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-10
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-10.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.95048754062838
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hLynA

Assay Title: Novascreen human unspecified LYN proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hLynA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hLynA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene LYN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hLynA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hLynA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hLynA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	20.885
Neutral control median absolute deviation, by plate:	0.570801
Positive control well median response value, by plate:	0.25
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	99.9125481084762
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.04210439921208
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hLynA_Activator

Assay Title: Novascreen human unspecified LYN proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hLynA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hLynA_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene LYN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hLynA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hLynA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hLynA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	20.885
Neutral control median absolute deviation, by plate:	0.570801
Positive control well median response value, by plate:	0.25
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	471.306631648063
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.04210439921208
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hLynB

Assay Title: Novascreen human unspecified LYN proto-oncogene, Src family tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hLynB was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hLynB, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene LYN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hLynB, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hLynB assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hLynB is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.91
Neutral control median absolute deviation, by plate:	0.800604
Positive control well median response value, by plate:	0.09
Positive control well median absolute deviation, by plate:	0.014826
Negative control well median, by plate:	100
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	3
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	29.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.42990534023934
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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Minoguchi K, Swaim WD, Berenstein EH, Siraganian RP. Src family tyrosine kinase p53/56lyn, a serine kinase and Fc epsilon RI associate with alpha-galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL-2H3 cells. J Biol Chem. 1994 Feb 18;269(7):5249-54. PubMed PMID: 8106508.|Sotirellis N, Johnson TM, Hibbs ML, Stanley IJ, Stanley E, Dunn AR, Cheng HC. Autophosphorylation induces autoactivation and a decrease in the Src homology 2 domain accessibility of the Lyn protein kinase. J Biol Chem. 1995 Dec 15;270(50):29773-80. PubMed PMID: 8530369.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Minoguchi K Swaim WD Berenstein EH Siraganian RP Src family tyrosine kinase p53 56lyn a serine kinase and Fc epsilon RI associate with alpha galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL 2H3 cells J Biol Chem 1994 Feb 18 269 7 5249 54 PubMed PMID 8106508 Sotirellis N Johnson TM Hibbs ML Stanley IJ Stanley E Dunn AR Cheng HC Autophosphorylation induces autoactivation and a decrease in the Src homology 2 domain accessibility of the Lyn protein kinase J Biol Chem 1995 Dec 15 270 50 29773 80 PubMed PMID 8530369 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Minoguchi K Swaim WD Berenstein EH Siraganian RP Src family tyrosine kinase p53 56lyn a serine kinase and Fc epsilon RI associate with alpha galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL 2H3 cells J Biol Chem 1994 Feb 18 269 7 5249 54 PubMed PMID 8106508 Sotirellis N

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Assay Title: Novascreen human unspecified LYN proto-oncogene, Src family tyrosine kinase assay

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Assay Summary:

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1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hLynB, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hLynB assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

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Assay Objectives:

NVS_ENZ_hLynB is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.91
Neutral control median absolute deviation, by plate:	0.800604
Positive control well median response value, by plate:	0.09
Positive control well median absolute deviation, by plate:	0.014826
Negative control well median, by plate:	2.85765315234863
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	3
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	29.9999999999998 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.42990534023934
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Minoguchi K, Swaim WD, Berenstein EH, Siraganian RP. Src family tyrosine kinase p53/56lyn, a serine kinase and Fc epsilon RI associate with alpha-galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL-2H3 cells. J Biol Chem. 1994 Feb 18;269(7):5249-54. PubMed PMID: 8106508.|Sotirellis N, Johnson TM, Hibbs ML, Stanley IJ, Stanley E, Dunn AR, Cheng HC. Autophosphorylation induces autoactivation and a decrease in the Src homology 2 domain accessibility of the Lyn protein kinase. J Biol Chem. 1995 Dec 15;270(50):29773-80. PubMed PMID: 8530369.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Minoguchi K Swaim WD Berenstein EH Siraganian RP Src family tyrosine kinase p53 56lyn a serine kinase and Fc epsilon RI associate with alpha galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL 2H3 cells J Biol Chem 1994 Feb 18 269 7 5249 54 PubMed PMID 8106508 Sotirellis N Johnson TM Hibbs ML Stanley IJ Stanley E Dunn AR Cheng HC Autophosphorylation induces autoactivation and a decrease in the Src homology 2 domain accessibility of the Lyn protein kinase J Biol Chem 1995 Dec 15 270 50 29773 80 PubMed PMID 8530369 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Minoguchi K Swaim WD Berenstein EH Siraganian RP Src family tyrosine kinase p53 56lyn a serine kinase and Fc epsilon RI associate with alpha galactosyl derivatives of ganglioside GD1b in rat basophilic leukemia RBL 2H3 cells J Biol Chem 1994 Feb 18 269 7 5249 54 PubMed PMID 8106508 Sotirellis N

Johnson TM Hibbs ML Stanley IJ Stanley E Dunn AR Cheng HC Autophosphorylation induces autoactivation and a decrease in the Src homology 2 domain accessibility of the Lyn protein kinase J Biol Chem 1995 Dec 15 270 50 29773 80 PubMed PMID 8530369 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPK1

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPK1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17.75
Neutral control median absolute deviation, by plate:	0.126021
Positive control well median response value, by plate:	0.51
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	100
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-74
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-139.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.41824957167333
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPK1_Activator

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPK1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17.75
Neutral control median absolute deviation, by plate:	0.126021
Positive control well median response value, by plate:	0.51
Positive control well median absolute deviation, by plate:	0.133434
Negative control well median, by plate:	14.0198909207571
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-74
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-139.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.41824957167333
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPK3

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPK3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPK3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPK3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPK3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPK3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	25.72
Neutral control median absolute deviation, by plate:	0.207564
Positive control well median response value, by plate:	1.68
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	96.2469135802469
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-60
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-98.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.62472552232692
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPK3_Activator

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPK3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPK3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPK3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPK3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPK3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	25.72
Neutral control median absolute deviation, by plate:	0.207564
Positive control well median response value, by plate:	1.68
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	33.0234438156831
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-60
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-98.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.62472552232692
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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FBS, Fetal Bovine Serum

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPK11

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPK11 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPK11, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK11. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPK11, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPK11 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPK11 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.52
Neutral control median absolute deviation, by plate:	0.800604
Positive control well median response value, by plate:	0.4
Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	99.1384432560903
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-42
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-42.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.770811051693408
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Reddy EP, Smith MJ, Srinivasan A. Nucleotide sequence of Abelson murine leukemia virus genome: structural similarity of its transforming gene product to other onc gene products with tyrosine-specific kinase activity. Proc Natl Acad Sci U S A. 1983 Jun;80(12):3623-7. PubMed PMID: 6304726; PubMed Central PMCID: PMC394102.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

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SSMD, Strictly Standardized Mean Difference

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPK11_Activator

Assay Title: Novascreen human unspecified mitogen-activated protein kinase 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPK11 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPK11_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPK11. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPK11, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPK11 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Assay Objectives:

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Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	5.40701128936422
Negative control well median absolute deviation value, by plate:	unspecified
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Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-42
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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References:

Reddy EP, Smith MJ, Srinivasan A. Nucleotide sequence of Abelson murine leukemia virus genome: structural similarity of its transforming gene product to other onc gene products with tyrosine-specific kinase activity. Proc Natl Acad Sci U S A. 1983 Jun;80(12):3623-7. PubMed PMID: 6304726; PubMed Central PMCID: PMC394102.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPKAPK2

Assay Title: Novascreen human unspecified mitogen-activated protein kinase-activated protein kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPKAPK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPKAPK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPKAPK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPKAPK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPKAPK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPKAPK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.4175
Neutral control median absolute deviation, by plate:	0.511497
Positive control well median response value, by plate:	0.69
Positive control well median absolute deviation, by plate:	0.200151
Negative control well median, by plate:	99.0761336523987
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-50.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.82329603738036
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPKAPK2_Activator

Assay Title: Novascreen human unspecified mitogen-activated protein kinase-activated protein kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPKAPK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPKAPK2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPKAPK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPKAPK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPKAPK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPKAPK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.4175
Neutral control median absolute deviation, by plate:	0.511497
Positive control well median response value, by plate:	0.69
Positive control well median absolute deviation, by plate:	0.200151
Negative control well median, by plate:	29.4387479762547
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-50.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.82329603738036
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMAPKAPK5

Assay Title: Novascreen human unspecified mitogen-activated protein kinase-activated protein kinase 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPKAPK5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPKAPK5, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPKAPK5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPKAPK5, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPKAPK5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPKAPK5 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.59
Neutral control median absolute deviation, by plate:	0.622692
Positive control well median response value, by plate:	2
Positive control well median absolute deviation, by plate:	0.07413
Negative control well median, by plate:	105.749405233941
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-55
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-55.55
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.88054718477398
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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NVS_ENZ_hMAPKAPK5_Activator

Assay Title: Novascreen human unspecified mitogen-activated protein kinase-activated protein kinase 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMAPKAPK5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMAPKAPK5_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MAPKAPK5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMAPKAPK5, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMAPKAPK5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMAPKAPK5 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.59
Neutral control median absolute deviation, by plate:	0.622692
Positive control well median response value, by plate:	2
Positive control well median absolute deviation, by plate:	0.07413
Negative control well median, by plate:	8.14168649220196
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-55
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-55.55
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.88054718477398
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMARK1

Assay Title: Novascreen human unspecified MAP/microtubule affinity-regulating kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMARK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMARK1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MARK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMARK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMARK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMARK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	46.28
Neutral control median absolute deviation, by plate:	2.00151
Positive control well median response value, by plate:	0.09
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	98.8318690095847
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.65785942492013
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMARK1_Activator

Assay Title: Novascreen human unspecified MAP/microtubule affinity-regulating kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMARK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMARK1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MARK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMARK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMARK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMARK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	46.28
Neutral control median absolute deviation, by plate:	2.00151
Positive control well median response value, by plate:	0.09
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	10.3434504792332
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.65785942492013
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMet

Assay Title: Novascreen human unspecified MET proto-oncogene, receptor tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMet was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMet, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MET. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMet, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMet assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMet is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.905
Neutral control median absolute deviation, by plate:	0.303933
Positive control well median response value, by plate:	0.22
Positive control well median absolute deviation, by plate:	0.066717
Negative control well median, by plate:	103.027877817794
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-89
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-92.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.70429365340857
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMet_Activator

Assay Title: Novascreen human unspecified MET proto-oncogene, receptor tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMet was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMet_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MET. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMet, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMet assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMet is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.905
Neutral control median absolute deviation, by plate:	0.303933
Positive control well median response value, by plate:	0.22
Positive control well median absolute deviation, by plate:	0.066717
Negative control well median, by plate:	14.7392290249433
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-89
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-92.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.70429365340857
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

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availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP1

Assay Title: Novascreen human unspecified matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9745
Neutral control median absolute deviation, by plate:	80.0604
Positive control well median response value, by plate:	3467
Positive control well median absolute deviation, by plate:	103.782
Negative control well median, by plate:	105.511393746688
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-42
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-80.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.37
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.41089209281765
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Knight CG, Willenbrock F, Murphy G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metalloproteinases. FEBS Lett. 1992 Jan 27;296(3):263-6. PubMed PMID: 1537400.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP1_Activator

Assay Title: Novascreen human unspecified matrix metallopeptidase 1 (interstitial collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9745
Neutral control median absolute deviation, by plate:	80.0604
Positive control well median response value, by plate:	3467
Positive control well median absolute deviation, by plate:	103.782
Negative control well median, by plate:	3172.21755211836
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-42
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-80.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

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Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP2

Assay Title: Novascreen human unspecified matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7701.5
Neutral control median absolute deviation, by plate:	108.23
Positive control well median response value, by plate:	3365.5
Positive control well median absolute deviation, by plate:	31.1346
Negative control well median, by plate:	99.6067545685866
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-61
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.44
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.51774784482759
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Knight CG, Willenbrock F, Murphy G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metalloproteinases. FEBS Lett. 1992 Jan 27;296(3):263-6. PubMed PMID: 1537400.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP2_Activator

Assay Title: Novascreen human unspecified matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7701.5
Neutral control median absolute deviation, by plate:	108.23
Positive control well median response value, by plate:	3365.5
Positive control well median absolute deviation, by plate:	31.1346
Negative control well median, by plate:	30.7888040712468
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-61
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-62.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.44
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.51774784482759
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP3

Assay Title: Novascreen human unspecified matrix metallopeptidase 3 (stromelysin 1, progelatinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	22871
Neutral control median absolute deviation, by plate:	241.664
Positive control well median response value, by plate:	5440
Positive control well median absolute deviation, by plate:	98.5929
Negative control well median, by plate:	105.848424708509
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-46
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-65.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.17511022701446
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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ER, Estrogen Receptor

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MIE, Molecular Initiating Event

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UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

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NVS_ENZ_hMMP3_Activator

Assay Title: Novascreen human unspecified matrix metallopeptidase 3 (stromelysin 1, progelatinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

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2. Assay Component Descriptions

Assay Objectives:

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Positive control well median response value, by plate:	5440
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Negative control well median, by plate:	34.4583848736526
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-46
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-65.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.17511022701446
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Knight CG, Willenbrock F, Murphy G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metalloproteinases. FEBS Lett. 1992 Jan 27;296(3):263-6. PubMed PMID: 1537400.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP7

Assay Title: Novascreen human unspecified matrix metallopeptidase 7 (matrilysin, uterine) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP7 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP7, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP7. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP7, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP7 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP7 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	15872
Neutral control median absolute deviation, by plate:	141.588
Positive control well median response value, by plate:	5626.5
Positive control well median absolute deviation, by plate:	59.304
Negative control well median, by plate:	104.948356807512
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-83.19
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.785587594507488
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Knight CG, Willenbrock F, Murphy G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metalloproteinases. FEBS Lett. 1992 Jan 27;296(3):263-6. PubMed PMID: 1537400.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP7_Activator

Assay Title: Novascreen human unspecified matrix metallopeptidase 7 (matrilysin, uterine) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP7 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP7_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP7. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP7, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP7 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP7 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	15872
Neutral control median absolute deviation, by plate:	141.588
Positive control well median response value, by plate:	5626.5
Positive control well median absolute deviation, by plate:	59.304
Negative control well median, by plate:	19.9943197955126
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-83.19
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.785587594507488
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Knight CG, Willenbrock F, Murphy G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metalloproteinases. FEBS Lett. 1992 Jan 27;296(3):263-6. PubMed PMID: 1537400.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP9

Assay Title: Novascreen human unspecified matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP9 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP9, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP9, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP9 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP9 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10813
Neutral control median absolute deviation, by plate:	139.364
Positive control well median response value, by plate:	3890.5
Positive control well median absolute deviation, by plate:	40.7159
Negative control well median, by plate:	288.972433709635
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-56
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-66.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.4
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.80479894370093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.8287936622056

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Bickett DM, Green MD, Berman J, Dezube M, Howe AS, Brown PJ, Roth JT, McGeehan GM. A high throughput fluorogenic substrate for interstitial collagenase (MMP-1) and gelatinase (MMP-9). Anal Biochem. 1993 Jul;212(1):58-64. PubMed PMID: 8368516.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP9_Activator

Assay Title: Novascreen human unspecified matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP9 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP9_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP9, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP9 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP9 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10813
Neutral control median absolute deviation, by plate:	139.364
Positive control well median response value, by plate:	3890.5
Positive control well median absolute deviation, by plate:	40.7159
Negative control well median, by plate:	2090.16393442623
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-56
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-66.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.4
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.80479894370093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.8287936622056

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMMP13

Assay Title: Novascreen human unspecified matrix metallopeptidase 13 (collagenase 3) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP13 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP13, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP13. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP13, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP13 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP13 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13317
Neutral control median absolute deviation, by plate:	189.773
Positive control well median response value, by plate:	3859
Positive control well median absolute deviation, by plate:	62.2692
Negative control well median, by plate:	115.9375
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-49
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-55.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.36680040288026
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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SSMD, Strictly Standardized Mean Difference

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5. Potential Regulatory Applications

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NVS_ENZ_hMMP13_Activator

Assay Title: Novascreen human unspecified matrix metallopeptidase 13 (collagenase 3) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMMP13 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMMP13_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MMP13. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "protease" intended target family, where the subfamily is "matrix metalloproteinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMMP13, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMMP13 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMMP13 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13317
Neutral control median absolute deviation, by plate:	189.773
Positive control well median response value, by plate:	3859
Positive control well median absolute deviation, by plate:	62.2692
Negative control well median, by plate:	481.758422350041
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-49
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-55.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.36680040288026
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMsk1

Assay Title: Novascreen human unspecified ribosomal protein S6 kinase, 90kDa, polypeptide 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMsk1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMsk1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene RPS6KA5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMsk1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMsk1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMsk1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19.82
Neutral control median absolute deviation, by plate:	0.326172
Positive control well median response value, by plate:	0.4
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	99.6971958786468
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-58.9
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.1089175855638
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hMsk1_Activator

Assay Title: Novascreen human unspecified ribosomal protein S6 kinase, 90kDa, polypeptide 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hMsk1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hMsk1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene RPS6KA5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hMsk1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hMsk1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hMsk1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19.82
Neutral control median absolute deviation, by plate:	0.326172
Positive control well median response value, by plate:	0.4
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	11.5253411306043
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-54
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-58.9
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.1089175855638
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hNEK2

Assay Title: Novascreen human unspecified NIMA-related kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hNEK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hNEK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene NEK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hNEK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hNEK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hNEK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.78
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	3.63
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	112.869080779944
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-106
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-200.73
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.28327504467093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hNEK2_Activator

Assay Title: Novascreen human unspecified NIMA-related kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hNEK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hNEK2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene NEK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hNEK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hNEK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hNEK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	27.78
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	3.63
Positive control well median absolute deviation, by plate:	0.059304
Negative control well median, by plate:	173.589626933576
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-106
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-200.73
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.28327504467093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPAK2

Assay Title: Novascreen human unspecified p21 protein (Cdc42/Rac)-activated kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPAK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPAK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PAK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPAK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPAK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPAK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19.34
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	0.12
Positive control well median absolute deviation, by plate:	0.088956
Negative control well median, by plate:	99.7880072683223
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-111
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-129.64
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.763301029678974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPAK2_Activator

Assay Title: Novascreen human unspecified p21 protein (Cdc42/Rac)-activated kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPAK2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPAK2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PAK2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPAK2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPAK2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPAK2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	19.34
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	0.12
Positive control well median absolute deviation, by plate:	0.088956
Negative control well median, by plate:	15.8994548758328
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-111
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-129.64
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.763301029678974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPAK4

Assay Title: Novascreen human unspecified p21 protein (Cdc42/Rac)-activated kinase 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPAK4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPAK4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PAK4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPAK4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPAK4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPAK4 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.615
Neutral control median absolute deviation, by plate:	0.859908
Positive control well median response value, by plate:	0.515
Positive control well median absolute deviation, by plate:	0.29652
Negative control well median, by plate:	107.437106918239
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-45
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.92
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.69030463334549
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPAK4_Activator

Assay Title: Novascreen human unspecified p21 protein (Cdc42/Rac)-activated kinase 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPAK4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPAK4_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PAK4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPAK4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPAK4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPAK4 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.615
Neutral control median absolute deviation, by plate:	0.859908
Positive control well median response value, by plate:	0.515
Positive control well median absolute deviation, by plate:	0.29652
Negative control well median, by plate:	8.80628830269118
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-45
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.92
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.69030463334549
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE1A1

Assay Title: Novascreen human unspecified phosphodiesterase 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE1A1_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE1B1

Assay Title: Novascreen human unspecified phosphodiesterase 1B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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unspecified

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DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE1B1_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 1B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE2A

Assay Title: Novascreen human unspecified phosphodiesterase 2A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE2A_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 2A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE3A

Assay Title: Novascreen human unspecified phosphodiesterase 3A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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NVS_ENZ_hPDE3A_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 3A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4A1

Assay Title: Novascreen human unspecified phosphodiesterase 4A, cAMP-specific assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPDE4A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPDE4A1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PDE4A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "phosphodiesterase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPDE4A1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPDE4A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPDE4A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.46
Neutral control median absolute deviation, by plate:	0.22239
Positive control well median response value, by plate:	1.3
Positive control well median absolute deviation, by plate:	0.252042
Negative control well median, by plate:	101.978184507303
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-102
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-184.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.38015619095604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.2809371457362

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4A1_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 4A, cAMP-specific assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPDE4A1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPDE4A1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PDE4A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "phosphodiesterase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPDE4A1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPDE4A1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPDE4A1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.46
Neutral control median absolute deviation, by plate:	0.22239
Positive control well median response value, by plate:	1.3
Positive control well median absolute deviation, by plate:	0.252042
Negative control well median, by plate:	22.5
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-102
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-184.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.38015619095604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.2809371457362

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4B1

Assay Title: Novascreen human unspecified phosphodiesterase 4B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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NVS_ENZ_hPDE4B1_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 4B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4C1

Assay Title: Novascreen human unspecified phosphodiesterase 4C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4C1_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 4C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4D3

Assay Title: Novascreen human unspecified phosphodiesterase 4D assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE4D3_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 4D assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE5

Assay Title: Novascreen human unspecified phosphodiesterase 5A, cGMP-specific assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPDE5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPDE5, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PDE5A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "phosphodiesterase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPDE5, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPDE5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPDE5 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.695
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	3.47
Positive control well median absolute deviation, by plate:	0.37065
Negative control well median, by plate:	111.027519303108
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-49
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-64.2
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.55317680882902
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE5_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 5A, cGMP-specific assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPDE5 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPDE5_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PDE5A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "phosphodiesterase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPDE5, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPDE5 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPDE5 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	34.695
Neutral control median absolute deviation, by plate:	0.533736
Positive control well median response value, by plate:	3.47
Positive control well median absolute deviation, by plate:	0.37065
Negative control well median, by plate:	20.8610567514677
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-49
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-64.2
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.55317680882902
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE7A

Assay Title: Novascreen human unspecified phosphodiesterase 7A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE7A_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 7A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE8A1

Assay Title: Novascreen human unspecified phosphodiesterase 8A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE8A1_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 8A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE9A2

Assay Title: Novascreen human unspecified phosphodiesterase 9A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE9A2_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 9A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE10

Assay Title: Novascreen human unspecified phosphodiesterase 10A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPDE10 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPDE10, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PDE10A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "phosphodiesterase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPDE10, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPDE10 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPDE10 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	49.83
Neutral control median absolute deviation, by plate:	0.978516
Positive control well median response value, by plate:	1.8
Positive control well median absolute deviation, by plate:	1.28986
Negative control well median, by plate:	99.6720663659403
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-56.82
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.10681460341897
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPDE10_Activator

Assay Title: Novascreen human unspecified phosphodiesterase 10A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPDE10 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPDE10_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PDE10A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "phosphodiesterase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPDE10, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPDE10 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPDE10 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	49.83
Neutral control median absolute deviation, by plate:	0.978516
Positive control well median response value, by plate:	1.8
Positive control well median absolute deviation, by plate:	1.28986
Negative control well median, by plate:	27.4361400189215
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-56.82
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.10681460341897
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPI3Ka

Assay Title: Novascreen human unspecified phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPI3Ka was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPI3Ka, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PIK3CA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "phosphoinositol kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPI3Ka, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPI3Ka assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPI3Ka is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.96
Neutral control median absolute deviation, by plate:	1.28986
Positive control well median response value, by plate:	1.54
Positive control well median absolute deviation, by plate:	0.29652
Negative control well median, by plate:	113.493661888402
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.14872637883688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.8923582730213

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPI3Ka_Activator

Assay Title: Novascreen human unspecified phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPI3Ka was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPI3Ka_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PIK3CA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "phosphoinositol kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPI3Ka, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPI3Ka assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPI3Ka is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.96
Neutral control median absolute deviation, by plate:	1.28986
Positive control well median response value, by plate:	1.54
Positive control well median absolute deviation, by plate:	0.29652
Negative control well median, by plate:	55.2040348464007
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.14872637883688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.8923582730213

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPKA

Assay Title: Novascreen human unspecified protein kinase, cAMP-dependent, catalytic, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPKA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPKA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKACA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPKA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPKA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPKA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.98
Neutral control median absolute deviation, by plate:	0.726474
Positive control well median response value, by plate:	0.45
Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	99.8156537221662
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-53.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.34826388888889
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPKA_Activator

Assay Title: Novascreen human unspecified protein kinase, cAMP-dependent, catalytic, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPKA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPKA_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKACA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPKA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPKA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPKA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	36.98
Neutral control median absolute deviation, by plate:	0.726474
Positive control well median response value, by plate:	0.45
Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	8.91218872870251
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-52
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-53.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.34826388888889
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

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Davies SP, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J. 2000 Oct 1;351(Pt 1):95-105. PubMed PMID: 10998351; PubMed Central PMCID: PMC1221339.|Flockhart, D.A. and Corbin, J.D.(1984) Brain Receptor Methodologies, Part A, Chapter 12 - PREPARATION OF THE CATALYTIC SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE. Academic Press, Orlando: 209-215|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPKCz

Assay Title: Novascreen human unspecified protein kinase C, zeta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPKCz was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPKCz, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKCZ. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPKCz, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPKCz assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPKCz is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.615
Neutral control median absolute deviation, by plate:	4.04009
Positive control well median response value, by plate:	2.885
Positive control well median absolute deviation, by plate:	2.1127
Negative control well median, by plate:	97.367581365667
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.36
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-7.11
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPKCz_Activator

Assay Title: Novascreen human unspecified protein kinase C, zeta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPKCz was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPKCz_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKCZ. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPKCz, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPKCz assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPKCz is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.615
Neutral control median absolute deviation, by plate:	4.04009
Positive control well median response value, by plate:	2.885
Positive control well median absolute deviation, by plate:	2.1127
Negative control well median, by plate:	13.4971282705807
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.36
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-7.11
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.59005583918316
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPKD2

Assay Title: Novascreen human unspecified protein kinase D2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPKD2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPKD2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKD2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPKD2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPKD2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPKD2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	49.225
Neutral control median absolute deviation, by plate:	11.3048
Positive control well median response value, by plate:	2.005
Positive control well median absolute deviation, by plate:	1.34175
Negative control well median, by plate:	99.6472184531884
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.2
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.56239620081411
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPKD2_Activator

Assay Title: Novascreen human unspecified protein kinase D2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPKD2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPKD2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PRKD2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPKD2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPKD2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPKD2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	49.225
Neutral control median absolute deviation, by plate:	11.3048
Positive control well median response value, by plate:	2.005
Positive control well median absolute deviation, by plate:	1.34175
Negative control well median, by plate:	7.12799638172773
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.2
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.56239620081411
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPM1A

Assay Title: Novascreen human unspecified protein phosphatase, Mg2+/Mn2+ dependent, 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPPM1A was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPPM1A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PPM1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "serine/threonine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPPM1A, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPPM1A assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPPM1A is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	43.44
Neutral control median absolute deviation, by plate:	0.555975
Positive control well median response value, by plate:	4.095
Positive control well median absolute deviation, by plate:	0.585627
Negative control well median, by plate:	98.5336856010568
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-51
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-79.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.27303830911492
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPM1A_Activator

Assay Title: Novascreen human unspecified protein phosphatase, Mg2+/Mn2+ dependent, 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPPM1A was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPPM1A_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PPM1A. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "serine/threonine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPPM1A, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPPM1A assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPPM1A is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	43.44
Neutral control median absolute deviation, by plate:	0.555975
Positive control well median response value, by plate:	4.095
Positive control well median absolute deviation, by plate:	0.585627
Negative control well median, by plate:	82.5231175693527
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-51
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-79.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.27303830911492
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPP1CA

Assay Title: Novascreen human unspecified protein phosphatase 1, catalytic subunit, alpha isozyme assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPPP1CA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPPP1CA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PPP1CA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "serine/threonine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPPP1CA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPPP1CA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPPP1CA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30.57
Neutral control median absolute deviation, by plate:	0.415128
Positive control well median response value, by plate:	6.19
Positive control well median absolute deviation, by plate:	0.489258
Negative control well median, by plate:	98.4780630233458
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-73.59
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.22
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.13704402277938
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.8222641366763

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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References:

Sogawa K, Yamada T, Oka S, Kawasaki K, Mori S, Tanaka H, Norimatsu H, Cai Y, Kuwabara H, Shima H, et al. Enhanced expression of catalytic subunit isoform PP1 gamma 1 of protein phosphatase type 1 associated with malignancy of osteogenic tumor. Cancer Lett. 1995 Feb 10;89(1):1-6. PubMed PMID: 7882291.|Hubbard MJ, Cohen P. On target with a new mechanism for the regulation of protein phosphorylation. Trends Biochem Sci. 1993 May;18(5):172-7. Review. PubMed PMID: 8392229.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Sogawa K Yamada T Oka S Kawasaki K Mori S Tanaka H Norimatsu H Cai Y Kuwabara H Shima H et al Enhanced expression of catalytic subunit isoform PP1 gamma 1 of protein phosphatase type 1 associated with malignancy of osteogenic tumor Cancer Lett 1995 Feb 10 89 1 1 6 PubMed PMID 7882291 Hubbard MJ Cohen P On target with a new mechanism for the regulation of protein phosphorylation Trends Biochem Sci 1993 May 18 5 172 7 Review PubMed PMID 8392229 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sogawa K Yamada T Oka S Kawasaki K Mori S Tanaka H Norimatsu H Cai Y Kuwabara H Shima H et al Enhanced expression of catalytic subunit isoform PP1 gamma 1 of protein phosphatase type 1 associated with malignancy of osteogenic tumor Cancer Lett 1995 Feb 10 89 1 1 6 PubMed PMID 7882291 Hubbard MJ Cohen P On target with a new mechanism for the regulation of protein phosphorylation Trends Biochem Sci 1993 May 18 5 172 7 Review PubMed PMID 8392229 Sipes NS

Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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<u>Contact Information:</u>

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPP1CA_Activator

Assay Title: Novascreen human unspecified protein phosphatase 1, catalytic subunit, alpha isozyme assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPPP1CA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPPP1CA_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PPP1CA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "serine/threonine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPPP1CA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPPP1CA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPPP1CA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	30.57
Neutral control median absolute deviation, by plate:	0.415128
Positive control well median response value, by plate:	6.19
Positive control well median absolute deviation, by plate:	0.489258
Negative control well median, by plate:	97.5909305621162
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-73.59
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.22
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.13704402277938
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.8222641366763

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sogawa K, Yamada T, Oka S, Kawasaki K, Mori S, Tanaka H, Norimatsu H, Cai Y, Kuwabara H, Shima H, et al. Enhanced expression of catalytic subunit isoform PP1 gamma 1 of protein phosphatase type 1 associated with malignancy of osteogenic tumor. Cancer Lett. 1995 Feb 10;89(1):1-6. PubMed PMID: 7882291.|Hubbard MJ, Cohen P. On target with a new mechanism for the regulation of protein phosphorylation. Trends Biochem Sci. 1993 May;18(5):172-7. Review. PubMed PMID: 8392229.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Sogawa K Yamada T Oka S Kawasaki K Mori S Tanaka H Norimatsu H Cai Y Kuwabara H Shima H et al Enhanced expression of catalytic subunit isoform PP1 gamma 1 of protein phosphatase type 1 associated with malignancy of osteogenic tumor Cancer Lett 1995 Feb 10 89 1 1 6 PubMed PMID 7882291 Hubbard MJ Cohen P On target with a new mechanism for the regulation of protein phosphorylation Trends Biochem Sci 1993 May 18 5 172 7 Review PubMed PMID 8392229 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Sogawa K Yamada T Oka S Kawasaki K Mori S Tanaka H Norimatsu H Cai Y Kuwabara H Shima H et al Enhanced expression of catalytic subunit isoform PP1 gamma 1 of protein phosphatase type 1 associated with malignancy of osteogenic tumor Cancer Lett 1995 Feb 10 89 1 1 6 PubMed

PMID 7882291 Hubbard MJ Cohen P On target with a new mechanism for the regulation of protein phosphorylation Trends Biochem Sci 1993 May 18 5 172 7 Review PubMed PMID 8392229 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPP2CA

Assay Title: Novascreen human unspecified protein phosphatase 2, catalytic subunit, alpha isozyme assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPPP2CA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPPP2CA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PPP2CA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "serine/threonine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPPP2CA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPPP2CA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPPP2CA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.6
Neutral control median absolute deviation, by plate:	1.27504
Positive control well median response value, by plate:	3.23
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	88.2216494845361
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-17.53
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.58349450549452
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Anderson NG, Maller JL, Tonks NK, Sturgill TW. Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. Nature. 1990 Feb 15;343(6259):651-3. PubMed PMID: 2154696.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPP2CA_Activator

Assay Title: Novascreen human unspecified protein phosphatase 2, catalytic subunit, alpha isozyme assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPPP2CA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPPP2CA_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PPP2CA. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "serine/threonine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPPP2CA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPPP2CA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPPP2CA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.6
Neutral control median absolute deviation, by plate:	1.27504
Positive control well median response value, by plate:	3.23
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	19.1752577319588
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-17.53
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.58349450549452
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPPP3CA

Assay Title: Novascreen human unspecified protein phosphatase 3 catalytic subunit alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTEN

Assay Title: Novascreen human unspecified phosphatase and tensin homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTEN was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTEN, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTEN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "dual-specific phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTEN, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTEN assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTEN is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.14
Neutral control median absolute deviation, by plate:	0.607866
Positive control well median response value, by plate:	10.5575
Positive control well median absolute deviation, by plate:	8.85112
Negative control well median, by plate:	128.571428571429
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-50.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.57445859348268
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.4467515608961

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTEN_Activator

Assay Title: Novascreen human unspecified phosphatase and tensin homolog assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTEN was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTEN_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTEN. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "dual-specific phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTEN, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTEN assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTEN is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.14
Neutral control median absolute deviation, by plate:	0.607866
Positive control well median response value, by plate:	10.5575
Positive control well median absolute deviation, by plate:	8.85112
Negative control well median, by plate:	384.928552170396
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-50.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.57445859348268
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.4467515608961

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN1

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.665
Neutral control median absolute deviation, by plate:	1.31951
Positive control well median response value, by plate:	2.5
Positive control well median absolute deviation, by plate:	0.096369
Negative control well median, by plate:	99.3752745161196
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.3
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.2273858651613
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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FBS, Fetal Bovine Serum

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NCI, Normalized Cell Index

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NVS_ENZ_hPTPN1_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 1 assay

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1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.665
Neutral control median absolute deviation, by plate:	1.31951
Positive control well median response value, by plate:	2.5
Positive control well median absolute deviation, by plate:	0.096369
Negative control well median, by plate:	260.221465076661
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.3
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.2273858651613
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Charbonneau H, Tonks NK. 1002 protein phosphatases? Annu Rev Cell Biol. 1992;8:463-93. Review. PubMed PMID: 1335746.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN2

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	23.23
Neutral control median absolute deviation, by plate:	1.36399
Positive control well median response value, by plate:	2.69
Positive control well median absolute deviation, by plate:	0.044478
Negative control well median, by plate:	103.537624230951
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-13.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.51404049775756
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.0842429865454

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gordon JA. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. Methods Enzymol. 1991;201:477-82. Review. PubMed PMID: 1943774.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Gordon JA Use of vanadate as protein phosphotyrosine phosphatase inhibitor Methods Enzymol 1991 201 477 82 Review PubMed PMID 1943774 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN2_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	23.23
Neutral control median absolute deviation, by plate:	1.36399
Positive control well median response value, by plate:	2.69
Positive control well median absolute deviation, by plate:	0.044478
Negative control well median, by plate:	371.032934131736
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-13.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.51404049775756
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.0842429865454

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Assay Source Information:

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN4

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 4 (megakaryocyte) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN4 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	41.22
Neutral control median absolute deviation, by plate:	0.919212
Positive control well median response value, by plate:	7.145
Positive control well median absolute deviation, by plate:	0.177912
Negative control well median, by plate:	102.808988764045
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-26
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-32.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.84157419779329
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.0494451867597

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN4_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 4 (megakaryocyte) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN4 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN4_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN4, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN4 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN4 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	41.22
Neutral control median absolute deviation, by plate:	0.919212
Positive control well median response value, by plate:	7.145
Positive control well median absolute deviation, by plate:	0.177912
Negative control well median, by plate:	101.057579318449
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-26
SSMD (median across all plates, using negative control wells):	unspecified
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Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.84157419779329
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.0494451867597

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN6

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN6, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN6, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN6 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.52
Neutral control median absolute deviation, by plate:	0.318759
Positive control well median response value, by plate:	8.545
Positive control well median absolute deviation, by plate:	0.126021
Negative control well median, by plate:	119.800399201597
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-73
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-92.13
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.90793690150479
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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AOP, Adverse Outcome Pathway

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Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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NVS_ENZ_hPTPN6_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN6 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN6_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN6, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN6 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

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Assay Objectives:

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Negative control well median absolute deviation value, by plate:	unspecified
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Z’ (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-92.13
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN9

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN9 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN9, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN9, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN9 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN9 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24
Neutral control median absolute deviation, by plate:	0.489258
Positive control well median response value, by plate:	2.58
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	101.104613385315
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.95334204648976
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN9_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 9 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN9 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN9_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN9. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN9, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN9 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN9 is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24
Neutral control median absolute deviation, by plate:	0.489258
Positive control well median response value, by plate:	2.58
Positive control well median absolute deviation, by plate:	0.22239
Negative control well median, by plate:	136.608187134503
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.95334204648976
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN11

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 11 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN11 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN11, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN11. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN11, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN11 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN11 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	44.55
Neutral control median absolute deviation, by plate:	0.66717
Positive control well median response value, by plate:	3.88
Positive control well median absolute deviation, by plate:	0.207564
Negative control well median, by plate:	115.832691814216
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-51.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.59230665770006
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN11 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN11_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN11. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN11, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN11 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN11 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	44.55
Neutral control median absolute deviation, by plate:	0.66717
Positive control well median response value, by plate:	3.88
Positive control well median absolute deviation, by plate:	0.207564
Negative control well median, by plate:	171.619194251989
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-51.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.59230665770006
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN12

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 12 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN12 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN12, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN12. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN12, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN12 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN12 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.595
Neutral control median absolute deviation, by plate:	0.756126
Positive control well median response value, by plate:	2.82
Positive control well median absolute deviation, by plate:	0.229803
Negative control well median, by plate:	103.756345177665
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-40
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-63.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.03999693613927
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Gordon JA. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. Methods Enzymol. 1991;201:477-82. Review. PubMed PMID: 1943774.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Gordon JA Use of vanadate as protein phosphotyrosine phosphatase inhibitor Methods Enzymol 1991 201 477 82 Review PubMed PMID 1943774 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN12_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 12 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN12 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN12_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN12. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN12, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN12 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN12 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.595
Neutral control median absolute deviation, by plate:	0.756126
Positive control well median response value, by plate:	2.82
Positive control well median absolute deviation, by plate:	0.229803
Negative control well median, by plate:	52.0848573518654
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-40
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-63.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.03999693613927
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN13

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN13 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN13, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN13. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN13, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN13 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN13 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	33.9
Neutral control median absolute deviation, by plate:	0.385476
Positive control well median response value, by plate:	11.57
Positive control well median absolute deviation, by plate:	0.578214
Negative control well median, by plate:	249.375
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-52.07
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.64749999999998
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN13_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN13 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN13_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN13. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN13, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN13 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN13 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	33.9
Neutral control median absolute deviation, by plate:	0.385476
Positive control well median response value, by plate:	11.57
Positive control well median absolute deviation, by plate:	0.578214
Negative control well median, by plate:	26.5542907180385
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-52.07
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.64749999999998
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN14

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 14 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN14 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN14, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN14. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN14, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN14 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPN14 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0.5 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	35.37
Neutral control median absolute deviation, by plate:	0.429954
Positive control well median response value, by plate:	2.76
Positive control well median absolute deviation, by plate:	0.177912
Negative control well median, by plate:	99.2753100928059
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-47
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.80333622936577
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPN14_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, non-receptor type 14 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPN14 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPN14_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPN14. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "non-receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPN14, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPN14 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

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Assay Objectives:

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Positive control well median absolute deviation, by plate:	0.177912
Negative control well median, by plate:	13.3479212253829
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-47
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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3.2 Assay Performance

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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRB

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRB was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRB, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRB, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRB assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRB is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32.1
Neutral control median absolute deviation, by plate:	0.37065
Positive control well median response value, by plate:	2.475
Positive control well median absolute deviation, by plate:	0.311346
Negative control well median, by plate:	101.198871650212
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-59
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-76.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.23184960798289
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Charbonneau H, Tonks NK. 1002 protein phosphatases? Annu Rev Cell Biol. 1992;8:463-93. Review. PubMed PMID: 1335746.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRB_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRB was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRB_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRB, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRB assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRB is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	32.1
Neutral control median absolute deviation, by plate:	0.37065
Positive control well median response value, by plate:	2.475
Positive control well median absolute deviation, by plate:	0.311346
Negative control well median, by plate:	33.1452750352609
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-59
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-76.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.23184960798289
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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AOP, Adverse Outcome Pathway

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRC

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRC, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRC is a biochemical, single-readout assay that uses extracted gene-proteins from yeast cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.963
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	0.258
Positive control well median absolute deviation, by plate:	0.0044478
Negative control well median, by plate:	122.795823665893
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-80
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-140.97
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.25
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

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Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.30438307034025
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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Pacitti A, Stevis P, Evans M, Trowbridge I, Higgins TJ. High level expression and purification of the enzymatically active cytoplasmic region of human CD45 phosphatase from yeast. Biochim Biophys Acta. 1994 Jun 30;1222(2):277-86. PubMed PMID: 8031864.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Pacitti A Stevis P Evans M Trowbridge I Higgins TJ High level expression and purification of the enzymatically active cytoplasmic region of human CD45 phosphatase from yeast Biochim Biophys Acta 1994 Jun 30 1222 2 277 86 PubMed PMID 8031864 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Pacitti A Stevis P Evans M Trowbridge I Higgins TJ High level expression and purification of the enzymatically active cytoplasmic region of human CD45 phosphatase from yeast Biochim Biophys Acta 1994 Jun 30 1222 2 277 86 PubMed PMID 8031864 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

<u>Date of Assay Document Creation:</u>

25 September 2018

<u>Author of Revisions:</u>

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRC_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRC_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRC is a biochemical, single-readout assay that uses extracted gene-proteins from yeast cells in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.963
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	0.258
Positive control well median absolute deviation, by plate:	0.0044478
Negative control well median, by plate:	20.3907203907204
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-80
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-140.97
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.25
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.30438307034025
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRF

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, F assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRF was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRF, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRF. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRF, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRF assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRF is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.085
Neutral control median absolute deviation, by plate:	0.311346
Positive control well median response value, by plate:	2.42
Positive control well median absolute deviation, by plate:	0.140847
Negative control well median, by plate:	98.589340889219
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-66
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-71.96
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.84105194984786
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Gordon JA. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. Methods Enzymol. 1991;201:477-82. Review. PubMed PMID: 1943774.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Gordon JA Use of vanadate as protein phosphotyrosine phosphatase inhibitor Methods Enzymol 1991 201 477 82 Review PubMed PMID 1943774 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRF_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, F assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRF was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRF_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRF. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRF, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRF assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

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Negative control well median absolute deviation value, by plate:	unspecified
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SSMD (median across all plates, using positive control wells):	-66
SSMD (median across all plates, using negative control wells):	unspecified
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Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
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Biological Response:

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All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.84105194984786
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gordon JA. Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. Methods Enzymol. 1991;201:477-82. Review. PubMed PMID: 1943774.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Gordon JA Use of vanadate as protein phosphotyrosine phosphatase inhibitor Methods Enzymol 1991 201 477 82 Review PubMed PMID 1943774 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRM

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, M assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRM was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRM, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRM. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRM, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRM assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRM is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.05
Neutral control median absolute deviation, by plate:	0.289107
Positive control well median response value, by plate:	2.505
Positive control well median absolute deviation, by plate:	0.200151
Negative control well median, by plate:	100.39751552795
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-74
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-114.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.3993046208951
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hPTPRM_Activator

Assay Title: Novascreen human unspecified protein tyrosine phosphatase, receptor type, M assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hPTPRM was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hPTPRM_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTPRM. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "phosphatase" intended target family, where the subfamily is "receptor tyrosine phosphatase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hPTPRM, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hPTPRM assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hPTPRM is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1.33 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.05
Neutral control median absolute deviation, by plate:	0.289107
Positive control well median response value, by plate:	2.505
Positive control well median absolute deviation, by plate:	0.200151
Negative control well median, by plate:	18.6378466557912
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-74
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-114.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.3993046208951
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hRAF1

Assay Title: Novascreen human unspecified Raf-1 proto-oncogene, serine/threonine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hRAF1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hRAF1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene RAF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hRAF1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hRAF1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hRAF1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.95
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	1.11
Positive control well median absolute deviation, by plate:	0.029652
Negative control well median, by plate:	101.795020266358
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-87
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-117.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.2610956825625
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

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AOP, Adverse Outcome Pathway

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hRAF1_Activator

Assay Title: Novascreen human unspecified Raf-1 proto-oncogene, serine/threonine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hRAF1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hRAF1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene RAF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hRAF1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hRAF1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hRAF1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.95
Neutral control median absolute deviation, by plate:	0.14826
Positive control well median response value, by plate:	1.11
Positive control well median absolute deviation, by plate:	0.029652
Negative control well median, by plate:	14.734693877551
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-87
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-117.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.2610956825625
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hROCK1

Assay Title: Novascreen human unspecified Rho-associated, coiled-coil containing protein kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hROCK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hROCK1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ROCK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hROCK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hROCK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hROCK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.6
Neutral control median absolute deviation, by plate:	0.711648
Positive control well median response value, by plate:	0.42
Positive control well median absolute deviation, by plate:	0.207564
Negative control well median, by plate:	99.1044266536781
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-50
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-53.14
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.19454415828545
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

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NVS_ENZ_hROCK1_Activator

Assay Title: Novascreen human unspecified Rho-associated, coiled-coil containing protein kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hROCK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hROCK1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ROCK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hROCK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hROCK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Assay Objectives:

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Assay Quality Statistics:

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Positive control well median response value, by plate:	0.42
Positive control well median absolute deviation, by plate:	0.207564
Negative control well median, by plate:	43.6195826645265
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-50
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-53.14
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Assay Performance Measures:

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Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

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EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSGK1

Assay Title: Novascreen human unspecified serum/glucocorticoid regulated kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSGK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSGK1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SGK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSGK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSGK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSGK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	40.815
Neutral control median absolute deviation, by plate:	3.26913
Positive control well median response value, by plate:	0.41
Positive control well median absolute deviation, by plate:	0.274281
Negative control well median, by plate:	104.357516995431
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-12.36
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.941805416248746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSGK1_Activator

Assay Title: Novascreen human unspecified serum/glucocorticoid regulated kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSGK1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSGK1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SGK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "serine/threonine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSGK1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSGK1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSGK1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	40.815
Neutral control median absolute deviation, by plate:	3.26913
Positive control well median response value, by plate:	0.41
Positive control well median absolute deviation, by plate:	0.274281
Negative control well median, by plate:	18.2547642928786
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-12.36
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.941805416248746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSIRT1

Assay Title: Novascreen human unspecified sirtuin 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSIRT1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSIRT1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SIRT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "protein deacetylase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSIRT1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSIRT1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSIRT1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	24.455
Neutral control median absolute deviation, by plate:	0.88956
Positive control well median response value, by plate:	1.215
Positive control well median absolute deviation, by plate:	0.155673
Negative control well median, by plate:	103.3105622701
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-30.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.44199498463128
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.6519699077877

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSIRT1_Activator

Assay Title: Novascreen human unspecified sirtuin 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSIRT1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSIRT1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SIRT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "protein deacetylase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSIRT1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSIRT1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	0.88956
Positive control well median response value, by plate:	1.215
Positive control well median absolute deviation, by plate:	0.155673
Negative control well median, by plate:	28.191249436175
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-30.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

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Standard minimum concentration tested:	0.0199999999999999 µM
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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSIRT2

Assay Title: Novascreen human unspecified sirtuin 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSIRT2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSIRT2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SIRT2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "protein deacetylase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSIRT2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSIRT2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSIRT2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.32
Neutral control median absolute deviation, by plate:	0.37065
Positive control well median response value, by plate:	1.87
Positive control well median absolute deviation, by plate:	0.237216
Negative control well median, by plate:	104.723799690243
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-75
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-127.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.44629278210776
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6777566926466

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSIRT2_Activator

Assay Title: Novascreen human unspecified sirtuin 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSIRT2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSIRT2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SIRT2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "protein deacetylase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSIRT2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSIRT2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSIRT2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.32
Neutral control median absolute deviation, by plate:	0.37065
Positive control well median response value, by plate:	1.87
Positive control well median absolute deviation, by plate:	0.237216
Negative control well median, by plate:	31.5723793677205
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-75
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-127.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.44629278210776
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6777566926466

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSIRT3

Assay Title: Novascreen human unspecified sirtuin 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSIRT3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSIRT3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SIRT3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "protein deacetylase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSIRT3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSIRT3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSIRT3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	42.685
Neutral control median absolute deviation, by plate:	2.23873
Positive control well median response value, by plate:	2.1
Positive control well median absolute deviation, by plate:	0.311346
Negative control well median, by plate:	98.5499542182413
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.63977594225016
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.838655653501

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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NVS_ENZ_hSIRT3_Activator

Assay Title: Novascreen human unspecified sirtuin 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSIRT3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSIRT3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SIRT3. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "hydrolase" intended target family, where the subfamily is "protein deacetylase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSIRT3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSIRT3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSIRT3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	42.685
Neutral control median absolute deviation, by plate:	2.23873
Positive control well median response value, by plate:	2.1
Positive control well median absolute deviation, by plate:	0.311346
Negative control well median, by plate:	40.2329749103943
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.63977594225016
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.838655653501

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSRC

Assay Title: Novascreen human unspecified SRC proto-oncogene, non-receptor tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSRC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSRC, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SRC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSRC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSRC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSRC is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.535
Neutral control median absolute deviation, by plate:	0.355824
Positive control well median response value, by plate:	0.405
Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	100.473153672084
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-88
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-102.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.36588146437606
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSRC_Activator

Assay Title: Novascreen human unspecified SRC proto-oncogene, non-receptor tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSRC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSRC_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SRC. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSRC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSRC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSRC is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	31.535
Neutral control median absolute deviation, by plate:	0.355824
Positive control well median response value, by plate:	0.405
Positive control well median absolute deviation, by plate:	0.14826
Negative control well median, by plate:	139.562087582484
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-88
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-102.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.36588146437606
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSyk

Assay Title: Novascreen human unspecified spleen tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSyk was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSyk, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SYK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSyk, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSyk assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSyk is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	56.6
Neutral control median absolute deviation, by plate:	1.1416
Positive control well median response value, by plate:	1.33
Positive control well median absolute deviation, by plate:	0.237216
Negative control well median, by plate:	99.0245664739884
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-47
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-48.41
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.99037933526012
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.9422760115607

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hSyk_Activator

Assay Title: Novascreen human unspecified spleen tyrosine kinase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hSyk was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hSyk_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene SYK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hSyk, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hSyk assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hSyk is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	56.6
Neutral control median absolute deviation, by plate:	1.1416
Positive control well median response value, by plate:	1.33
Positive control well median absolute deviation, by plate:	0.237216
Negative control well median, by plate:	27.9624277456647
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-47
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-48.41
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.99037933526012
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.9422760115607

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hTie2

Assay Title: Novascreen human unspecified TEK tyrosine kinase, endothelial assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hTie2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hTie2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene TEK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hTie2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hTie2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hTie2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.31
Neutral control median absolute deviation, by plate:	0.281694
Positive control well median response value, by plate:	0.64
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	101.999370277078
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-82
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-84.6
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.2800092838241
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hTie2_Activator

Assay Title: Novascreen human unspecified TEK tyrosine kinase, endothelial assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hTie2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hTie2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene TEK. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hTie2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hTie2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hTie2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	29.31
Neutral control median absolute deviation, by plate:	0.281694
Positive control well median response value, by plate:	0.64
Positive control well median absolute deviation, by plate:	0.118608
Negative control well median, by plate:	11.1150193457615
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-82
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-84.6
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

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Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hTrkA

Assay Title: Novascreen human unspecified neurotrophic tyrosine kinase, receptor, type 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hTrkA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hTrkA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene NTRK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hTrkA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hTrkA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hTrkA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.345
Neutral control median absolute deviation, by plate:	0.874734
Positive control well median response value, by plate:	0.71
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	100.455788514129
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.98
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.6248449690126
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hTrkA_Activator

Assay Title: Novascreen human unspecified neurotrophic tyrosine kinase, receptor, type 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hTrkA was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hTrkA_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene NTRK1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hTrkA, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hTrkA assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hTrkA is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	37.345
Neutral control median absolute deviation, by plate:	0.874734
Positive control well median response value, by plate:	0.71
Positive control well median absolute deviation, by plate:	0.163086
Negative control well median, by plate:	67.9943100995733
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.98
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.6248449690126
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hVEGFR1

Assay Title: Novascreen human unspecified fms-related tyrosine kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hVEGFR1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hVEGFR1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FLT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hVEGFR1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hVEGFR1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hVEGFR1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	25.755
Neutral control median absolute deviation, by plate:	0.622692
Positive control well median response value, by plate:	0.455
Positive control well median absolute deviation, by plate:	0.185325
Negative control well median, by plate:	104.228855721393
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-33
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.87846251605537
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.2707750963322

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hVEGFR1_Activator

Assay Title: Novascreen human unspecified fms-related tyrosine kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hVEGFR1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hVEGFR1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FLT1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hVEGFR1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hVEGFR1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hVEGFR1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	0.622692
Positive control well median response value, by plate:	0.455
Positive control well median absolute deviation, by plate:	0.185325
Negative control well median, by plate:	170.955574182733
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-33
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.87846251605537
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.2707750963322

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hVEGFR2

Assay Title: Novascreen human unspecified kinase insert domain receptor (a type III receptor tyrosine kinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hVEGFR2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hVEGFR2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene KDR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hVEGFR2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hVEGFR2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hVEGFR2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.75
Neutral control median absolute deviation, by plate:	0.311346
Positive control well median response value, by plate:	2
Positive control well median absolute deviation, by plate:	0.192738
Negative control well median, by plate:	101.611627447103
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-88
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-110.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.2444005903919
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hVEGFR2_Activator

Assay Title: Novascreen human unspecified kinase insert domain receptor (a type III receptor tyrosine kinase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hVEGFR2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hVEGFR2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene KDR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hVEGFR2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hVEGFR2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hVEGFR2 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	38.75
Neutral control median absolute deviation, by plate:	0.311346
Positive control well median response value, by plate:	2
Positive control well median absolute deviation, by plate:	0.192738
Negative control well median, by plate:	71.3031735313977
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.95
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-88
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-110.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.2444005903919
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hVEGFR3

Assay Title: Novascreen human unspecified fms-related tyrosine kinase 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hVEGFR3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hVEGFR3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FLT4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hVEGFR3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hVEGFR3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hVEGFR3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.9
Neutral control median absolute deviation, by plate:	0.681996
Positive control well median response value, by plate:	0.41
Positive control well median absolute deviation, by plate:	0.029652
Negative control well median, by plate:	104.100827689992
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-43.42
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.35664881948814
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hVEGFR3_Activator

Assay Title: Novascreen human unspecified fms-related tyrosine kinase 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hVEGFR3 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hVEGFR3_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene FLT4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hVEGFR3, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hVEGFR3 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hVEGFR3 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26.9
Neutral control median absolute deviation, by plate:	0.681996
Positive control well median response value, by plate:	0.41
Positive control well median absolute deviation, by plate:	0.029652
Negative control well median, by plate:	14.25548287803
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-43
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-43.42
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.35664881948814
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hZAP70

Assay Title: Novascreen human unspecified zeta-chain (TCR) associated protein kinase 70kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hZAP70 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hZAP70, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ZAP70. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hZAP70, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hZAP70 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hZAP70 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.13
Neutral control median absolute deviation, by plate:	0.66717
Positive control well median response value, by plate:	2
Positive control well median absolute deviation, by plate:	1.34917
Negative control well median, by plate:	102.766323024055
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-39.17
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5.5
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	38.7298334620741 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.08861340206185
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.5316804123711

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_hZAP70_Activator

Assay Title: Novascreen human unspecified zeta-chain (TCR) associated protein kinase 70kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_hZAP70 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_hZAP70_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene ZAP70. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "kinase" intended target family, where the subfamily is "non-receptor tyrosine kinase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_ENZ_hZAP70, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_hZAP70 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with fluorescence intensity signals by Fluorescence and electrophoretic mobility shift technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_hZAP70 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 0 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	28.13
Neutral control median absolute deviation, by plate:	0.66717
Positive control well median response value, by plate:	2
Positive control well median absolute deviation, by plate:	1.34917
Negative control well median, by plate:	4.84536082474227
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-39.17
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5.5
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	38.7298334620741 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.08861340206185
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Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_oCOX1

Assay Title: Novascreen sheep unspecified prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_oCOX1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_oCOX1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTGS1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "cyclooxygenase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_oCOX1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_oCOX1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_oCOX1 is a biochemical, single-readout assay that uses extracted gene-proteins from Ovine seminal vesicles in a tissue-based cell-free assay.Measurements were taken 0.66 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.156
Neutral control median absolute deviation, by plate:	0.0014826
Positive control well median response value, by plate:	0.086
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	3772.8125
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-67
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-96.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.43
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.24814159292036
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.4888495575222

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_oCOX1_Activator

Assay Title: Novascreen sheep unspecified prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_oCOX1 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_oCOX1_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTGS1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "cyclooxygenase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_oCOX1, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_oCOX1 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_oCOX1 is a biochemical, single-readout assay that uses extracted gene-proteins from Ovine seminal vesicles in a tissue-based cell-free assay.Measurements were taken 0.66 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.156
Neutral control median absolute deviation, by plate:	0.0014826
Positive control well median response value, by plate:	0.086
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	2225.56390977444
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.96
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-67
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-96.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.43
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.24814159292036
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Chemical Library Scope and Limitations:

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zweifel BS, Shaffer A, Talley JJ, Masferrer JL, Seibert K, Isakson PC. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci U S A. 1998 Oct 27;95(22):13313-8. PubMed PMID: 9789085; PubMed Central PMCID: PMC23795.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Smith CJ Zhang Y Koboldt CM Muhammad J Zweifel BS Shaffer A Talley JJ Masferrer JL Seibert K Isakson PC Pharmacological analysis of cyclooxygenase 1 in inflammation Proc Natl Acad Sci U S A 1998 Oct 27 95 22 13313 8 PubMed PMID 9789085 PubMed Central PMCID PMC23795 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Smith CJ Zhang Y Koboldt CM Muhammad J Zweifel BS Shaffer A Talley JJ Masferrer JL Seibert K Isakson PC Pharmacological analysis of cyclooxygenase 1 in inflammation Proc Natl Acad Sci U S A 1998 Oct 27 95 22 13313 8 PubMed PMID 9789085 PubMed Central PMCID PMC23795 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS

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Masferrer JL Seibert K Isakson PC Pharmacological analysis of cyclooxygenase 1 in inflammation Proc Natl Acad Sci U S A 1998 Oct 27 95 22 13313 8 PubMed PMID 9789085 PubMed Central PMCID PMC23795 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Masferrer JL Seibert K Isakson PC Pharmacological analysis of cyclooxygenase 1 in inflammation Proc Natl Acad Sci U S A 1998 Oct 27 95 22 13313 8 PubMed PMID 9789085 PubMed Central PMCID PMC23795 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_oCOX2

Assay Title: Novascreen sheep unspecified prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_oCOX2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_oCOX2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTGS2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "cyclooxygenase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_oCOX2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_oCOX2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_oCOX2 is a biochemical, single-readout assay that uses extracted gene-proteins from Ovine placenta in a tissue-based cell-free assay.Measurements were taken 0.66 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.153
Neutral control median absolute deviation, by plate:	0.0014826
Positive control well median response value, by plate:	0.061
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	98.7951807228916
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-94
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-93.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.41
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.893132530120482
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Kargman S, Wong E, Greig GM, Falgueyret JP, Cromlish W, Ethier D, Yergey JA, Riendeau D, Evans JF, Kennedy B, Tagari P, Francis DA, O'Neill GP. Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and -2 in intact cells. Biochem Pharmacol. 1996 Oct 11;52(7):1113-25. PubMed PMID: 8831731.|Seibert K, Masferrer JL, Needleman P, Salvemini D. Pharmacological manipulation of cyclo-oxygenase-2 in the inflamed hydronephrotic kidney. Br J Pharmacol. 1996 Mar;117(6):1016-20. PubMed PMID: 8882591; PubMed Central PMCID: PMC1909763.|Rosenstock M, Danon A, Rimon G. PGHS-2 inhibitors, NS-398 and DuP-697, attenuate the inhibition of PGHS-1 by aspirin and indomethacin without altering its activity. Biochim Biophys Acta. 1999 Aug 25;1440(1):127-37. PubMed PMID: 10477832.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV

Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878

95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_oCOX2_Activator

Assay Title: Novascreen sheep unspecified prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_oCOX2 was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_oCOX2_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene PTGS2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "cyclooxygenase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_oCOX2, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_oCOX2 assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_oCOX2 is a biochemical, single-readout assay that uses extracted gene-proteins from Ovine placenta in a tissue-based cell-free assay.Measurements were taken 0.66 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.153
Neutral control median absolute deviation, by plate:	0.0014826
Positive control well median response value, by plate:	0.061
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	313.372093023256
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-94
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-93.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.41
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.893132530120482
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Kargman S, Wong E, Greig GM, Falgueyret JP, Cromlish W, Ethier D, Yergey JA, Riendeau D, Evans JF, Kennedy B, Tagari P, Francis DA, O'Neill GP. Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and -2 in intact cells. Biochem Pharmacol. 1996 Oct 11;52(7):1113-25. PubMed PMID: 8831731.|Seibert K, Masferrer JL, Needleman P, Salvemini D. Pharmacological manipulation of cyclo-oxygenase-2 in the inflamed hydronephrotic kidney. Br J Pharmacol. 1996 Mar;117(6):1016-20. PubMed PMID: 8882591; PubMed Central PMCID: PMC1909763.|Rosenstock M, Danon A, Rimon G. PGHS-2 inhibitors, NS-398 and DuP-697, attenuate the inhibition of PGHS-1 by aspirin and indomethacin without altering its activity. Biochim Biophys Acta. 1999 Aug 25;1440(1):127-37. PubMed PMID: 10477832.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Kargman S Wong E Greig GM Falgueyret JP Cromlish W Ethier D Yergey JA Riendeau D Evans JF Kennedy B Tagari P Francis DA O Neill GP Mechanism of selective inhibition of human prostaglandin G H synthase 1 and 2 in intact cells Biochem Pharmacol 1996 Oct 11 52 7 1113 25 PubMed PMID 8831731 Seibert K Masferrer JL Needleman P Salvemini D Pharmacological manipulation of cyclo oxygenase 2 in the inflamed hydronephrotic kidney Br J Pharmacol 1996 Mar 117 6 1016 20 PubMed PMID 8882591 PubMed Central PMCID PMC1909763 Rosenstock M Danon A Rimon G PGHS 2 inhibitors NS 398 and DuP 697 attenuate the inhibition of PGHS 1 by aspirin and indomethacin without altering its activity Biochim Biophys Acta 1999 Aug 25 1440 1 127 37 PubMed PMID 10477832 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_pMTHFR

Assay Title: Novascreen pig unspecified methylenetetrahydrofolate reductase (NAD(P)H) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_pMTHFR was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_pMTHFR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MTHFR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "methylenetetrahydrofolate reductase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_pMTHFR, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_pMTHFR assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_pMTHFR is a biochemical, single-readout assay that uses extracted gene-proteins from Porcine kidney in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9760.36
Neutral control median absolute deviation, by plate:	293.095
Positive control well median response value, by plate:	2929.18
Positive control well median absolute deviation, by plate:	53.9444
Negative control well median, by plate:	77.1228452377664
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

model

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.1294626179889
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.7767757079334

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Matthews RG, Haywood BJ. Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate: some mechanistic and regulatory implications. Biochemistry. 1979 Oct 30;18(22):4845-51. PubMed PMID: 508720.|Suormala T, Gamse G, Fowler B. 5,10-Methylenetetrahydrofolate reductase (MTHFR) assay in the forward direction: residual activity in MTHFR deficiency. Clin Chem. 2002 Jun;48(6 Pt 1):835-43. PubMed PMID: 12028998.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_pMTHFR_Activator

Assay Title: Novascreen pig unspecified methylenetetrahydrofolate reductase (NAD(P)H) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_pMTHFR was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_pMTHFR_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene MTHFR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "methylenetetrahydrofolate reductase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_pMTHFR, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_pMTHFR assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_pMTHFR is a biochemical, single-readout assay that uses extracted gene-proteins from Porcine kidney in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9760.36
Neutral control median absolute deviation, by plate:	293.095
Positive control well median response value, by plate:	2929.18
Positive control well median absolute deviation, by plate:	53.9444
Negative control well median, by plate:	31.9939414624571
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.29
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

model

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.1294626179889
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.7767757079334

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Matthews RG, Haywood BJ. Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate: some mechanistic and regulatory implications. Biochemistry. 1979 Oct 30;18(22):4845-51. PubMed PMID: 508720.|Suormala T, Gamse G, Fowler B. 5,10-Methylenetetrahydrofolate reductase (MTHFR) assay in the forward direction: residual activity in MTHFR deficiency. Clin Chem. 2002 Jun;48(6 Pt 1):835-43. PubMed PMID: 12028998.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt

1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51

PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Matthews RG Haywood BJ Inhibition of pig liver methylenetetrahydrofolate reductase by dihydrofolate some mechanistic and regulatory implications Biochemistry 1979 Oct 30 18 22 4845 51 PubMed PMID 508720 Suormala T Gamse G Fowler B 5 10 Methylenetetrahydrofolate reductase MTHFR assay in the forward direction residual activity in MTHFR deficiency Clin Chem 2002 Jun 48 6 Pt 1 835 43 PubMed PMID 12028998 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rabI2C

Assay Title: Novascreen rabbit unspecified creatine kinase, brain assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rabI2C was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rabI2C, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CKB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "imidazoline receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rabI2C, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rabI2C assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rabI2C is a biochemical, single-readout assay that uses extracted gene-proteins from Rabbit brain membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3866.89
Neutral control median absolute deviation, by plate:	87.2732
Positive control well median response value, by plate:	182.74
Positive control well median absolute deviation, by plate:	11.7051
Negative control well median, by plate:	101.064081768493
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-39
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-43.58
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.99637411257512
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Brown CM, MacKinnon AC, McGrath JC, Spedding M, Kilpatrick AT. Alpha 2-adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. Br J Pharmacol. 1990 Apr;99(4):803-9. PubMed PMID: 1972896; PubMed Central PMCID: PMC1917565.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Brown CM MacKinnon AC McGrath JC Spedding M Kilpatrick AT Alpha 2 adrenoceptor subtypes and imidazoline like binding sites in the rat brain Br J Pharmacol 1990 Apr 99 4 803 9 PubMed PMID 1972896 PubMed Central PMCID PMC1917565 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Brown CM MacKinnon AC McGrath JC Spedding M Kilpatrick AT Alpha 2 adrenoceptor subtypes and imidazoline like binding sites in the rat brain Br J Pharmacol 1990 Apr 99 4 803 9 PubMed PMID 1972896 PubMed Central PMCID PMC1917565 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rabI2C_Activator

Assay Title: Novascreen rabbit unspecified creatine kinase, brain assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rabI2C was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rabI2C_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene CKB. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "imidazoline receptor". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rabI2C, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rabI2C assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rabI2C is a biochemical, single-readout assay that uses extracted gene-proteins from Rabbit brain membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3866.89
Neutral control median absolute deviation, by plate:	87.2732
Positive control well median response value, by plate:	182.74
Positive control well median absolute deviation, by plate:	11.7051
Negative control well median, by plate:	25.4700581550187
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.92
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-39
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-43.58
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.99637411257512
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Brown CM, MacKinnon AC, McGrath JC, Spedding M, Kilpatrick AT. Alpha 2-adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. Br J Pharmacol. 1990 Apr;99(4):803-9. PubMed PMID: 1972896; PubMed Central PMCID: PMC1917565.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rACFSKBinding

Assay Title: Novascreen rat unspecified adenylate cyclase 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rACFSKBinding was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rACFSKBinding, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Adcy5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "lyase" intended target family, where the subfamily is "adenylyl cyclase".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rACFSKBinding, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rACFSKBinding assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rACFSKBinding is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3451.19
Neutral control median absolute deviation, by plate:	90.6017
Positive control well median response value, by plate:	746.32
Positive control well median absolute deviation, by plate:	25.6712
Negative control well median, by plate:	99.8291747931939
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-22
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-24.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.68357486891746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.1014492135048

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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References:

Seamon KB, Daly JW. High-affinity binding of forskolin to rat brain membranes. Adv Cyclic Nucleotide Protein Phosphorylation Res. 1985;19:125-35. PubMed PMID: 3159185.|Seamon KB, Vaillancourt R, Edwards M, Daly JW. Binding of [3H]forskolin to rat brain membranes. Proc Natl Acad Sci U S A. 1984 Aug;81(16):5081-5. PubMed PMID: 6433343; PubMed Central PMCID: PMC391641.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Seamon KB Daly JW High affinity binding of forskolin to rat brain membranes Adv Cyclic Nucleotide Protein Phosphorylation Res 1985 19 125 35 PubMed PMID 3159185 Seamon KB Vaillancourt R Edwards M Daly JW Binding of 3H forskolin to rat brain membranes Proc Natl Acad Sci U S A 1984 Aug 81 16 5081 5 PubMed PMID 6433343 PubMed Central PMCID PMC391641 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Seamon KB Daly JW High affinity binding of forskolin to rat brain membranes Adv Cyclic Nucleotide Protein Phosphorylation Res 1985 19 125 35 PubMed PMID 3159185 Seamon KB Vaillancourt R Edwards M Daly JW Binding of 3H forskolin to rat brain membranes Proc Natl Acad Sci U S A 1984 Aug 81 16 5081 5 PubMed PMID 6433343 PubMed Central PMCID PMC391641 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rACFSKBinding_Activator

Assay Title: Novascreen rat unspecified adenylate cyclase 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rACFSKBinding was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rACFSKBinding_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Adcy5. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "lyase" intended target family, where the subfamily is "adenylyl cyclase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rACFSKBinding, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rACFSKBinding assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rACFSKBinding is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3451.19
Neutral control median absolute deviation, by plate:	90.6017
Positive control well median response value, by plate:	746.32
Positive control well median absolute deviation, by plate:	25.6712
Negative control well median, by plate:	226.266180008301
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-22
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-24.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.68357486891746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.1014492135048

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Seamon KB, Daly JW. High-affinity binding of forskolin to rat brain membranes. Adv Cyclic Nucleotide Protein Phosphorylation Res. 1985;19:125-35. PubMed PMID: 3159185.|Seamon KB, Vaillancourt R, Edwards M, Daly JW. Binding of [3H]forskolin to rat brain membranes. Proc Natl Acad Sci U S A. 1984 Aug;81(16):5081-5. PubMed PMID: 6433343; PubMed Central PMCID: PMC391641.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Seamon KB Daly JW High affinity binding of forskolin to rat brain membranes Adv Cyclic Nucleotide Protein Phosphorylation Res 1985 19 125 35 PubMed PMID 3159185 Seamon KB Vaillancourt R Edwards M Daly JW Binding of 3H forskolin to rat brain membranes Proc Natl Acad Sci U S A 1984 Aug 81 16 5081 5 PubMed PMID 6433343 PubMed Central PMCID PMC391641 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Seamon KB Daly JW High affinity binding of forskolin to rat brain membranes Adv Cyclic Nucleotide Protein Phosphorylation Res 1985 19 125 35 PubMed PMID 3159185 Seamon KB Vaillancourt R Edwards M Daly JW Binding of 3H forskolin to rat brain membranes Proc Natl Acad Sci U S A 1984 Aug 81 16 5081 5 PubMed PMID 6433343 PubMed Central PMCID PMC391641 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB

Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rAChE_Activator

Assay Title: Novascreen rat unspecified acetylcholinesterase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rAChE was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rAChE_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Ache. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "esterase" intended target family, where the subfamily is "acetylcholinesterase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rAChE, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rAChE assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by Colorimetric technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rAChE is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain membranes in a tissue-based cell-free assay.Measurements were taken 0.33 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.486
Neutral control median absolute deviation, by plate:	0.0029652
Positive control well median response value, by plate:	0.388
Positive control well median absolute deviation, by plate:	0.0051891
Negative control well median, by plate:	28.0276816608997
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.97
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-112
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-263.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.26
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.65150379746835
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

ELLMAN GL, COURTNEY KD, ANDRES V Jr, FEATHER-STONE RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul;7:88-95. PubMed PMID: 13726518.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rCNOS

Assay Title: Novascreen rat unspecified nitric oxide synthase 1, neuronal assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rCNOS was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rCNOS, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Nos1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "nitric oxide synthase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rCNOS, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rCNOS assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rCNOS is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10909.5
Neutral control median absolute deviation, by plate:	188.29
Positive control well median response value, by plate:	1858
Positive control well median absolute deviation, by plate:	66.717
Negative control well median, by plate:	120.647580095433
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36.76
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.11020576184513
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.6612345710708

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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References:

Michel AD, Phul RK, Stewart TL, Humphrey PP. Characterization of the binding of [3H]-L-NG-nitro-arginine in rat brain. Br J Pharmacol. 1993 Jun;109(2):287-8. PubMed PMID: 7689392; PubMed Central PMCID: PMC2175700.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Michel AD Phul RK Stewart TL Humphrey PP Characterization of the binding of 3H L NG nitro arginine in rat brain Br J Pharmacol 1993 Jun 109 2 287 8 PubMed PMID 7689392 PubMed Central PMCID PMC2175700 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Michel AD Phul RK Stewart TL Humphrey PP Characterization of the binding of 3H L NG nitro arginine in rat brain Br J Pharmacol 1993 Jun 109 2 287 8 PubMed PMID 7689392 PubMed Central PMCID PMC2175700 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rCOMT

Assay Title: Novascreen rat unspecified catechol-O-methyltransferase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rCOMT was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rCOMT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Comt. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "methyltransferase" intended target family, where the subfamily is "o-methyltransferase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rCOMT, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rCOMT assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by HPLC and Spectrophotometry technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rCOMT is a biochemical, single-readout assay that uses extracted gene-proteins from Rat dorsal striatum in a tissue-based cell-free assay. Measurements were taken 0 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	501.5
Neutral control median absolute deviation, by plate:	21.4977
Positive control well median response value, by plate:	0.5
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	97.4558670820353
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.8
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.34
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.33771015210153
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.0262609126092

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Assay Source Information:

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rCOMT_Activator

Assay Title: Novascreen rat unspecified catechol-O-methyltransferase assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rCOMT was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rCOMT_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Comt. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "methyltransferase" intended target family, where the subfamily is "o-methyltransferase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rCOMT, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rCOMT assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with absorbance signals by HPLC and Spectrophotometry technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rCOMT is a biochemical, single-readout assay that uses extracted gene-proteins from Rat dorsal striatum in a tissue-based cell-free assay.Measurements were taken 0 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	501.5
Neutral control median absolute deviation, by plate:	21.4977
Positive control well median response value, by plate:	0.5
Positive control well median absolute deviation, by plate:	0
Negative control well median, by plate:	50.1845018450185
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.34
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.33771015210153
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.0262609126092

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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References:

Smit NP, Pavel S, Kammeyer A, Westerhof W. Determination of catechol O-methyltransferase activity in relation to melanin metabolism using high-performance liquid chromatography with fluorimetric detection. Anal Biochem. 1990 Nov 1;190(2):286-91. PubMed PMID: 2291472.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Smit NP Pavel S Kammeyer A Westerhof W Determination of catechol O methyltransferase activity in relation to melanin metabolism using high performance liquid chromatography with fluorimetric detection Anal Biochem 1990 Nov 1 190 2 286 91 PubMed PMID 2291472 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Smit NP Pavel S Kammeyer A Westerhof W Determination of catechol O methyltransferase activity in relation to melanin metabolism using high performance liquid chromatography with fluorimetric detection Anal Biochem 1990 Nov 1 190 2 286 91 PubMed PMID 2291472 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOAC

Assay Title: Novascreen rat unspecified monoamine oxidase A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOAC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOAC, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maoa. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOAC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOAC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOAC is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7879.98
Neutral control median absolute deviation, by plate:	261.471
Positive control well median response value, by plate:	618.49
Positive control well median absolute deviation, by plate:	38.8145
Negative control well median, by plate:	103.432281594122
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-35
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.39
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.12466620760733
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

OTSUKA S KOBAYASHI Y RADIOISOTOPIC ASSAY FOR MONOAMINE OXIDASE DETERMINATIONS IN HUMAN PLASMA Biochem Pharmacol 1964 Jul 13 995 1006 PubMed PMID 14201142 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |OTSUKA S KOBAYASHI Y RADIOISOTOPIC ASSAY FOR MONOAMINE OXIDASE DETERMINATIONS IN HUMAN PLASMA Biochem Pharmacol 1964 Jul 13 995 1006 PubMed PMID 14201142 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOAC_Activator

Assay Title: Novascreen rat unspecified monoamine oxidase A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOAC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOAC_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maoa. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOAC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOAC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOAC is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7879.98
Neutral control median absolute deviation, by plate:	261.471
Positive control well median response value, by plate:	618.49
Positive control well median absolute deviation, by plate:	38.8145
Negative control well median, by plate:	46.3661414955981
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-35
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.39
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOAP

Assay Title: Novascreen rat unspecified monoamine oxidase A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOAP was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOAP, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maoa. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOAP, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOAP assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOAP is a biochemical, single-readout assay that uses extracted gene-proteins from Rat liver mitochondrial membranes in a tissue-based cell-free assay.Measurements were taken 1.17 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	8269.97
Neutral control median absolute deviation, by plate:	485.403
Positive control well median response value, by plate:	885
Positive control well median absolute deviation, by plate:	58.3255
Negative control well median, by plate:	103.45562887233
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.26183394029155
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.5710036417493

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOAP_Activator

Assay Title: Novascreen rat unspecified monoamine oxidase A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOAP was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOAP_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maoa. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOAP, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOAP assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOAP is a biochemical, single-readout assay that uses extracted gene-proteins from Rat liver mitochondrial membranes in a tissue-based cell-free assay.Measurements were taken 1.17 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	8269.97
Neutral control median absolute deviation, by plate:	485.403
Positive control well median response value, by plate:	885
Positive control well median absolute deviation, by plate:	58.3255
Negative control well median, by plate:	33.0279232111693
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.26183394029155
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.5710036417493

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOBC

Assay Title: Novascreen rat unspecified monoamine oxidase B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOBC was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOBC, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maob. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOBC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOBC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOBC is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain in a tissue-based cell-free assay.Measurements were taken 1.17 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5780.4
Neutral control median absolute deviation, by plate:	242.101
Positive control well median response value, by plate:	594.105
Positive control well median absolute deviation, by plate:	20.0522
Negative control well median, by plate:	106.361155141973
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.02
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.70696747542796
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.2418048525678

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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NVS_ENZ_rMAOBC_Activator

Assay Title: Novascreen rat unspecified monoamine oxidase B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

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1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOBC, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOBC assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOBC is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain in a tissue-based cell-free assay.Measurements were taken 1.17 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5780.4
Neutral control median absolute deviation, by plate:	242.101
Positive control well median response value, by plate:	594.105
Positive control well median absolute deviation, by plate:	20.0522
Negative control well median, by plate:	39.4656000713863
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.02
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.70696747542796
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.2418048525678

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

OTSUKA S KOBAYASHI Y RADIOISOTOPIC ASSAY FOR MONOAMINE OXIDASE DETERMINATIONS IN HUMAN PLASMA Biochem Pharmacol 1964 Jul 13 995 1006 PubMed PMID 14201142 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |OTSUKA S KOBAYASHI Y RADIOISOTOPIC ASSAY FOR MONOAMINE OXIDASE DETERMINATIONS IN HUMAN PLASMA Biochem Pharmacol 1964 Jul 13 995 1006 PubMed PMID 14201142 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOBP

Assay Title: Novascreen rat unspecified monoamine oxidase B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOBP was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOBP, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, loss-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maob. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOBP, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOBP assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOBP is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain in a tissue-based cell-free assay.Measurements were taken 1.17 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	8066.5
Neutral control median absolute deviation, by plate:	122.893
Positive control well median response value, by plate:	525.01
Positive control well median absolute deviation, by plate:	15.5376
Negative control well median, by plate:	109.427052369457
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.29698599430599
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

OTSUKA S KOBAYASHI Y RADIOISOTOPIC ASSAY FOR MONOAMINE OXIDASE DETERMINATIONS IN HUMAN PLASMA Biochem Pharmacol 1964 Jul 13 995 1006 PubMed PMID 14201142 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |OTSUKA S KOBAYASHI Y RADIOISOTOPIC ASSAY FOR MONOAMINE OXIDASE DETERMINATIONS IN HUMAN PLASMA Biochem Pharmacol 1964 Jul 13 995 1006 PubMed PMID 14201142 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_ENZ_rMAOBP_Activator

Assay Title: Novascreen rat unspecified monoamine oxidase B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_ENZ_rMAOBP was analyzed into 2 assay endpoints. This assay endpoint, NVS_ENZ_rMAOBP_Activator, was analyzed in the negative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of enzyme reporter, gain-of-signal activity can be used to understand changes in the enzymatic activity as they relate to the gene Maob. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a enzymatic activity function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "oxidoreductase" intended target family, where the subfamily is "monoamine oxidase". Assay was not developed or optimized to detect gain of signal. Use data with caution.

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_ENZ_rMAOBP, is one of 1 assay component(s) measured or calculated from the NVS_ENZ_rMAOBP assay. It is designed to make measurements of enzyme activity, a form of enzyme reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_ENZ_rMAOBP is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain in a tissue-based cell-free assay.Measurements were taken 1.17 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	8066.5
Neutral control median absolute deviation, by plate:	122.893
Positive control well median response value, by plate:	525.01
Positive control well median absolute deviation, by plate:	15.5376
Negative control well median, by plate:	26.4023388258156
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-48
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.29698599430599
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_bAdoR_NonSelective

Assay Title: Novascreen bovine unspecified adenosine A1 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_bAdoR_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_bAdoR_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADORA1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_bAdoR_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_bAdoR_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_bAdoR_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine striatal membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4339
Neutral control median absolute deviation, by plate:	119.349
Positive control well median response value, by plate:	635.5
Positive control well median absolute deviation, by plate:	25.9455
Negative control well median, by plate:	104.124381993234
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-27.77
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.44109665159449
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.6465799095669

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Holtzman SG, Mante S, Minneman KP. Role of adenosine receptors in caffeine tolerance. J Pharmacol Exp Ther. 1991 Jan;256(1):62-8. PubMed PMID: 1846425.|Weir RL, Anderson SM, Daly JW. Inhibition of N6-[3H]cyclohexyladenosine binding by carbamazepine. Epilepsia. 1990 Sep-Oct;31(5):503-12. PubMed PMID: 2401242.|Bruns RF, Lu GH, Pugsley TA. Characterization of the A2 adenosine receptor labeled by [3H]NECA in rat striatal membranes. Mol Pharmacol. 1986 Apr;29(4):331-46. PubMed PMID: 3010074.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_bAT2

Assay Title: Novascreen bovine unspecified angiotensin II receptor, type 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_bAT2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_bAT2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene AGTR2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_bAT2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_bAT2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_bAT2 is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine cerebellar membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5873
Neutral control median absolute deviation, by plate:	75.6126
Positive control well median response value, by plate:	948
Positive control well median absolute deviation, by plate:	25.9455
Negative control well median, by plate:	92.08301545824
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-49
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-57.8
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.29209972730814
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Bennett JP Jr, Snyder SH. Angiotensin II binding to mammalian brain membranes. J Biol Chem. 1976 Dec 10;251(23):7423-30. PubMed PMID: 187587.|Wiest SA, Rampersaud A, Zimmerman K, Steinberg MI. Characterization of distinct angiotensin II binding sites in rat adrenal gland and bovine cerebellum using selective nonpeptide antagonists. J Cardiovasc Pharmacol. 1991 Feb;17(2):177-84. PubMed PMID: 1709220.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Bennett JP Jr Snyder SH Angiotensin II binding to mammalian brain membranes J Biol Chem 1976 Dec 10 251 23 7423 30 PubMed PMID 187587 Wiest SA Rampersaud A Zimmerman K Steinberg MI Characterization of distinct angiotensin II binding sites in rat adrenal gland and bovine cerebellum using selective nonpeptide antagonists J Cardiovasc Pharmacol 1991 Feb 17 2 177 84 PubMed PMID 1709220 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_bDR_NonSelective

Assay Title: Novascreen bovine unspecified dopamine receptor D1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_bDR_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_bDR_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene DRD1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_bDR_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_bDR_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_bDR_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine striatal membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4492.46
Neutral control median absolute deviation, by plate:	146.785
Positive control well median response value, by plate:	505.685
Positive control well median absolute deviation, by plate:	30.3118
Negative control well median, by plate:	105.314296522087
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-29
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-30.87
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.04683662025719
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.2810197215431

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

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Leysen JE, Gommeren W, Laduron PM. Spiperone: a ligand of choice for neuroleptic receptors. 1. Kinetics and characteristics of in vitro binding. Biochem Pharmacol. 1978 Feb 1;27(3):307-16. PubMed PMID: 23126.|Creese I, Schneider R, Snyder SH. 3H-Spiroperidol labels dopamine receptors in pituitary and brain. Eur J Pharmacol. 1977 Dec 15;46(4):377-81. PubMed PMID: 590345.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Leysen JE Gommeren W Laduron PM Spiperone a ligand of choice for neuroleptic receptors 1 Kinetics and characteristics of in vitro binding Biochem Pharmacol 1978 Feb 1 27 3 307 16 PubMed PMID 23126 Creese I Schneider R Snyder SH 3H Spiroperidol labels dopamine receptors in pituitary and brain Eur J Pharmacol 1977 Dec 15 46 4 377 81 PubMed PMID 590345 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_bH1

Assay Title: Novascreen bovine unspecified histamine receptor H1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_bH1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_bH1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HRH1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_bH1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_bH1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_bH1 is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine cerebellar membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4032.04
Neutral control median absolute deviation, by plate:	119.023
Positive control well median response value, by plate:	930.21
Positive control well median absolute deviation, by plate:	46.8353
Negative control well median, by plate:	108.647938345704
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.40632248315475
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.4379348989285

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Chang RS, Tran VT, Snyder SH. Heterogeneity of histamine H1-receptors: species variations in [3H]mepyramine binding of brain membranes. J Neurochem. 1979 Jun;32(6):1653-63. PubMed PMID: 448359.|Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, Palacios JM. Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. Brain Res. 1990 Sep 3;526(2):322-7. PubMed PMID: 1979518.|Haaksma EE, Leurs R, Timmerman H. Histamine receptors: subclasses and specific ligands. Pharmacol Ther. 1990;47(1):73-104. Review. PubMed PMID: 2195558.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_bNPY_NonSelective

Assay Title: Novascreen bovine unspecified neuropeptide Y assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_bNPY_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_bNPY_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NPY. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_bNPY_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_bNPY_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_bNPY_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine hippocampal membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3005
Neutral control median absolute deviation, by plate:	142.33
Positive control well median response value, by plate:	754
Positive control well median absolute deviation, by plate:	57.8214
Negative control well median, by plate:	101.138475836431
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.76
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.25
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.00524031333188
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.0314418799913

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Martel JC, Fournier A, St Pierre S, Quirion R. Quantitative autoradiographic distribution of [125I]Bolton-Hunter neuropeptide Y receptor binding sites in rat brain. Comparison with [125I]peptide YY receptor sites. Neuroscience. 1990;36(1):255-83. PubMed PMID: 2170863.|Chang RS, Lotti VJ, Chen TB, Cerino DJ, Kling PJ. Neuropeptide Y (NPY) binding sites in rat brain labeled with 125I-Bolton-Hunter NPY: comparative potencies of various polypeptides on brain NPY binding and biological responses in the rat vas deferens. Life Sci. 1985 Dec 2;37(22):2111-22. PubMed PMID: 2999540.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Martel JC Fournier A St Pierre S Quirion R Quantitative autoradiographic distribution of 125I Bolton Hunter neuropeptide Y receptor binding sites in rat brain Comparison with 125I peptide YY receptor sites Neuroscience 1990 36 1 255 83 PubMed PMID 2170863 Chang RS Lotti VJ Chen TB Cerino DJ Kling PJ Neuropeptide Y NPY binding sites in rat brain labeled with 125I Bolton Hunter NPY comparative potencies of various polypeptides on brain NPY binding and biological responses in the rat vas deferens Life Sci 1985 Dec 2 37 22 2111 22 PubMed PMID 2999540 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_g5HT4

Assay Title: Novascreen guinea pig unspecified 5 hydroxytryptamine (serotonin) receptor 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_g5HT4 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_g5HT4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Htr4. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_g5HT4, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_g5HT4 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_g5HT4 is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig striatal membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2321.68
Neutral control median absolute deviation, by plate:	55.1972
Positive control well median response value, by plate:	544.085
Positive control well median absolute deviation, by plate:	22.6986
Negative control well median, by plate:	133.09858683052
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-26
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-34.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.60327201017373
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.6196320610424

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Grossman CJ, Kilpatrick GJ, Bunce KT. Development of a radioligand binding assay for 5-HT4 receptors in guinea-pig and rat brain. Br J Pharmacol. 1993 Jul;109(3):618-24. PubMed PMID: 8358562; PubMed Central PMCID: PMC2175660.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Grossman CJ Kilpatrick GJ Bunce KT Development of a radioligand binding assay for 5 HT4 receptors in guinea pig and rat brain Br J Pharmacol 1993 Jul 109 3 618 24 PubMed PMID 8358562 PubMed Central PMCID PMC2175660 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gANPA

Assay Title: Novascreen guinea pig unspecified natriuretic peptide A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gANPA was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gANPA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Nppa. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "lyase" intended target family, where the subfamily is "guanylyl cyclase".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gANPA, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gANPA assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gANPA is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig cerebellar membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3816.5
Neutral control median absolute deviation, by plate:	126.532
Positive control well median response value, by plate:	1737
Positive control well median absolute deviation, by plate:	56.3388
Negative control well median, by plate:	96.0516338176828
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.64
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-12.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.48
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

model

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.58545047762695
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.5127028657617

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Schiffrin EL, Chartier L, Thibault G, St-Louis J, Cantin M, Genest J. Vascular and adrenal receptors for atrial natriuretic factor in the rat. Circ Res. 1985 Jun;56(6):801-7. PubMed PMID: 2408776.|FÃ©thiÃ¨re J, Meloche S, Nguyen TT, Ong H, De Lean A. Distinct properties of atrial natriuretic factor receptor subpopulations in epithelial and fibroblast cell lines. Mol Pharmacol. 1989 May;35(5):584-92. PubMed PMID: 2542756.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Schiffrin EL Chartier L Thibault G St Louis J Cantin M Genest J Vascular and adrenal receptors for atrial natriuretic factor in the rat Circ Res 1985 Jun 56 6 801 7 PubMed PMID 2408776 F thi re J Meloche S Nguyen TT Ong H De Lean A Distinct properties of atrial natriuretic factor receptor subpopulations in epithelial and fibroblast cell lines Mol Pharmacol 1989 May 35 5 584 92 PubMed PMID 2542756 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Schiffrin EL Chartier L Thibault G St Louis J Cantin M Genest J Vascular and adrenal receptors for atrial natriuretic factor in the rat Circ Res 1985 Jun 56 6 801 7 PubMed PMID 2408776 F thi re J Meloche S Nguyen TT Ong H De Lean A Distinct properties of atrial natriuretic factor receptor subpopulations in epithelial and fibroblast cell lines Mol Pharmacol 1989 May 35 5 584 92 PubMed PMID 2542756 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID

PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949
Schiffrin EL Chartier L Thibault G St Louis J Cantin M Genest J Vascular and adrenal receptors for atrial natriuretic factor in the rat Circ Res 1985 Jun 56 6 801 7 PubMed PMID 2408776 F thi re J Meloche S Nguyen TT Ong H De Lean A Distinct properties of atrial natriuretic factor receptor subpopulations in epithelial and fibroblast cell lines Mol Pharmacol 1989 May 35 5 584 92 PubMed PMID 2542756
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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gBK2

Assay Title: Novascreen guinea pig unspecified bradykinin receptor, beta 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gBK2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gBK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Bdkrb2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gBK2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gBK2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gBK2 is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig ileum membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1481.98
Neutral control median absolute deviation, by plate:	46.094
Positive control well median response value, by plate:	238.72
Positive control well median absolute deviation, by plate:	13.0617
Negative control well median, by plate:	110.14800807046
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-22
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.96
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.73743103448276
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	40.4245862068966

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Ransom RW, Young GS, Schneck K, Goodman CB. Characterization of solubilized bradykinin B2 receptors from smooth muscle and mucosa of guinea pig ileum. Biochem Pharmacol. 1992 Apr 15;43(8):1823-7. PubMed PMID: 1315547.|Manning DC, Vavrek R, Stewart JM, Snyder SH. Two bradykinin binding sites with picomolar affinities. J Pharmacol Exp Ther. 1986 May;237(2):504-12. PubMed PMID: 2871174.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Ransom RW Young GS Schneck K Goodman CB Characterization of solubilized bradykinin B2 receptors from smooth muscle and mucosa of guinea pig ileum Biochem Pharmacol 1992 Apr 15 43 8 1823 7 PubMed PMID 1315547 Manning DC Vavrek R Stewart JM Snyder SH Two bradykinin binding sites with picomolar affinities J Pharmacol Exp Ther 1986 May 237 2 504 12 PubMed PMID 2871174 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Ransom RW Young GS Schneck K Goodman CB Characterization of solubilized bradykinin B2 receptors from smooth muscle and mucosa of guinea pig ileum Biochem Pharmacol 1992 Apr 15 43 8 1823 7 PubMed PMID 1315547 Manning DC Vavrek R Stewart JM Snyder SH Two bradykinin binding sites with picomolar affinities J Pharmacol Exp Ther 1986 May 237 2 504 12 PubMed PMID 2871174 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh

AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Ransom RW Young GS Schneck K Goodman CB Characterization of solubilized bradykinin B2 receptors from smooth muscle and mucosa of guinea pig ileum Biochem Pharmacol 1992 Apr 15 43 8 1823 7 PubMed PMID 1315547 Manning DC Vavrek R Stewart JM Snyder SH Two bradykinin binding sites with picomolar affinities J Pharmacol Exp Ther 1986 May 237 2 504 12 PubMed PMID 2871174 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Ransom RW Young GS Schneck K Goodman CB Characterization of solubilized bradykinin B2 receptors from smooth muscle and mucosa of guinea pig ileum Biochem Pharmacol 1992 Apr 15 43 8 1823 7 PubMed PMID 1315547 Manning DC Vavrek R Stewart JM Snyder SH Two bradykinin binding sites with picomolar affinities J Pharmacol Exp Ther 1986 May 237 2 504 12 PubMed PMID 2871174 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Ransom RW Young GS Schneck K Goodman CB Characterization of solubilized bradykinin B2 receptors from smooth muscle and mucosa of guinea pig ileum Biochem Pharmacol 1992 Apr 15 43 8 1823 7 PubMed PMID 1315547 Manning DC Vavrek R Stewart JM Snyder SH Two bradykinin binding sites with picomolar affinities J Pharmacol Exp Ther 1986 May 237 2 504 12 PubMed PMID 2871174 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gH2

Assay Title: Novascreen guinea pig unspecified histamine receptor H2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gH2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gH2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Hrh2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gH2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gH2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gH2 is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig striatal membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	15546
Neutral control median absolute deviation, by plate:	856.943
Positive control well median response value, by plate:	2743
Positive control well median absolute deviation, by plate:	106.747
Negative control well median, by plate:	115.312992407964
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.81
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.8
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.81701789929604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.9021073957762

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, Palacios JM. Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. Brain Res. 1990 Sep 3;526(2):322-7. PubMed PMID: 1979518.|Haaksma EE, Leurs R, Timmerman H. Histamine receptors: subclasses and specific ligands. Pharmacol Ther. 1990;47(1):73-104. Review. PubMed PMID: 2195558.|Gajtkowski GA, Norris DB, Rising TJ, Wood TP. Specific binding of 3H-tiotidine to histamine H2 receptors in guinea pig cerebral cortex. Nature. 1983 Jul 7-13;304(5921):65-7. PubMed PMID: 6135157.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Martinez Mir MI Pollard H Moreau J Arrang JM Ruat M Traiffort E Schwartz JC Palacios JM Three histamine receptors H1 H2 and H3 visualized in the brain of human and non human primates Brain Res 1990 Sep 3 526 2 322 7 PubMed PMID 1979518 Haaksma EE Leurs R Timmerman H Histamine receptors subclasses and specific ligands Pharmacol Ther 1990 47 1 73 104 Review PubMed PMID 2195558 Gajtkowski GA Norris DB Rising TJ Wood TP Specific binding of 3H tiotidine to histamine H2 receptors in guinea pig cerebral cortex Nature 1983 Jul 7 13 304 5921 65 7 PubMed PMID 6135157 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Martinez Mir MI Pollard H Moreau J Arrang JM Ruat M Traiffort E Schwartz JC Palacios JM Three histamine receptors H1 H2 and H3 visualized in the brain of human and non human primates Brain Res 1990 Sep 3 526 2 322 7 PubMed PMID 1979518 Haaksma EE Leurs R Timmerman H Histamine receptors subclasses and specific ligands Pharmacol Ther 1990 47 1 73 104

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gLTB4

Assay Title: Novascreen guinea pig unspecified leukotriene B4 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gLTB4 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gLTB4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Ltb4r. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gLTB4, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gLTB4 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gLTB4 is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig spleen membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2383.27
Neutral control median absolute deviation, by plate:	111.032
Positive control well median response value, by plate:	788.72
Positive control well median absolute deviation, by plate:	58.8147
Negative control well median, by plate:	127.174584018255
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.66
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.96161095497277
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.7696657298366

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gardiner PJ, Abram TS, Cuthbert NJ. Evidence for two leukotriene receptor types in the guinea-pig isolated ileum. Eur J Pharmacol. 1990 Jul 3;182(2):291-9. PubMed PMID: 2168834.|Cheng JB, Cheng EI, Kohi F, Townley RG. [3H]leukotriene B4 binding to the guinea-pig spleen membrane preparation: a rich tissue source for a high-affinity leukotriene B4 receptor site. J Pharmacol Exp Ther. 1986 Jan;236(1):126-32. PubMed PMID: 3001280.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gLTD4

Assay Title: Novascreen guinea pig unspecified cysteinyl leukotriene receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gLTD4 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gLTD4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cysltr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gLTD4, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gLTD4 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gLTD4 is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig lung membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	8772.11
Neutral control median absolute deviation, by plate:	218.165
Positive control well median response value, by plate:	664.73
Positive control well median absolute deviation, by plate:	51.7131
Negative control well median, by plate:	98.5301495143878
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.21
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.75211948720761
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.5127169232457

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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Norman P, Abram TS, Cuthbert NJ, Gardiner PJ. The inhibition of [3H]leukotriene D4 binding to guinea-pig lung membranes. The correlation of binding affinity with activity on the guinea-pig ileum. Eur J Pharmacol. 1990 Jul 3;182(2):301-12. PubMed PMID: 2397744.|Hogaboom GK, Mong S, Wu HL, Crooke ST. Peptidoleukotrienes: distinct receptors for leukotriene C4 and D4 in the guinea-pig lung. Biochem Biophys Res Commun. 1983 Nov 15;116(3):1136-43. PubMed PMID: 6316967.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Norman P Abram TS Cuthbert NJ Gardiner PJ The inhibition of 3H leukotriene D4 binding to guinea pig lung membranes The correlation of binding affinity with activity on the guinea pig ileum Eur J Pharmacol 1990 Jul 3 182 2 301 12 PubMed PMID 2397744 Hogaboom GK Mong S Wu HL Crooke ST Peptidoleukotrienes distinct receptors for leukotriene C4 and D4 in the guinea pig lung Biochem Biophys Res Commun 1983 Nov 15 116 3 1136 43 PubMed PMID 6316967 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Norman P Abram TS Cuthbert NJ Gardiner PJ The inhibition of 3H leukotriene D4 binding to guinea pig lung membranes The correlation of binding affinity with activity on the guinea pig ileum Eur J Pharmacol 1990 Jul 3 182 2 301 12 PubMed PMID 2397744 Hogaboom GK Mong S Wu HL Crooke ST Peptidoleukotrienes distinct receptors for leukotriene C4 and D4 in the guinea pig lung Biochem Biophys Res Commun 1983 Nov 15 116 3 1136 43 PubMed PMID 6316967 Sipes NS Martin MT

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

25 September 2018

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gMPeripheral_NonSelective

Assay Title: Novascreen guinea pig unspecified cholinergic receptor, muscarinic 3, cardiac assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gMPeripheral_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gMPeripheral_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Chrm3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gMPeripheral_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gMPeripheral_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gMPeripheral_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig bladder membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1642.8
Neutral control median absolute deviation, by plate:	62.1432
Positive control well median response value, by plate:	161.96
Positive control well median absolute deviation, by plate:	13.6029
Negative control well median, by plate:	111.656202580986
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-24.61
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.89460162313763
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.3676097388258

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Luthin GR, Wolfe BB. Comparison of [3H]pirenzepine and [3H]quinuclidinylbenzilate binding to muscarinic cholinergic receptors in rat brain. J Pharmacol Exp Ther. 1984 Mar;228(3):648-55. PubMed PMID: 6546770.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Luthin GR Wolfe BB Comparison of 3H pirenzepine and 3H quinuclidinylbenzilate binding to muscarinic cholinergic receptors in rat brain J Pharmacol Exp Ther 1984 Mar 228 3 648 55 PubMed PMID 6546770 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Luthin GR Wolfe BB Comparison of 3H pirenzepine and 3H quinuclidinylbenzilate binding to muscarinic cholinergic receptors in rat brain J Pharmacol Exp Ther 1984 Mar 228 3 648 55 PubMed PMID 6546770 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets

Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Luthin GR Wolfe BB Comparison of 3H pirenzepine and 3H quinuclidinylbenzilate binding to muscarinic cholinergic receptors in rat brain J Pharmacol Exp Ther 1984 Mar 228 3 648 55 PubMed PMID 6546770 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_gOpiateK

Assay Title: Novascreen guinea pig unspecified opioid receptor, kappa 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_gOpiateK was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_gOpiateK, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Oprk1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_gOpiateK, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_gOpiateK assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_gOpiateK is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig cerebellar membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1542.99
Neutral control median absolute deviation, by plate:	48.3476
Positive control well median response value, by plate:	145.43
Positive control well median absolute deviation, by plate:	8.99938
Negative control well median, by plate:	107.371947462753
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-30.85
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.85427323834633
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.125639430078

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Rothman RB, Bykov V, Xue BG, Xu H, De Costa BR, Jacobson AE, Rice KC, Kleinman JE, Brady LS. Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain. Evidence for species differences. Peptides. 1992 Sep-Oct;13(5):977-87. PubMed PMID: 1336192.|Kinouchi K, Pasternak GW. Evidence for kappa 1 opioid receptor multiplicity in the guinea pig cerebellum. Eur J Pharmacol. 1991 Jun 19;207(2):135-41. PubMed PMID: 1679014.|Lahti RA, Mickelson MM, McCall JM, Von Voigtlander PF. [3H]U-69593 a highly selective ligand for the opioid kappa receptor. Eur J Pharmacol. 1985 Feb 26;109(2):281-4. PubMed PMID: 2986999.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Rothman RB Bykov V Xue BG Xu H De Costa BR Jacobson AE Rice KC Kleinman JE Brady LS Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain Evidence for species differences Peptides 1992 Sep Oct 13 5 977 87 PubMed PMID 1336192 Kinouchi K Pasternak GW Evidence for kappa 1 opioid receptor multiplicity in the guinea pig cerebellum Eur J Pharmacol 1991 Jun 19 207 2 135 41 PubMed PMID 1679014 Lahti RA Mickelson MM McCall JM Von Voigtlander PF 3H U 69593 a highly selective ligand for the opioid kappa receptor Eur J Pharmacol 1985 Feb 26 109 2 281 4 PubMed PMID 2986999 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Rothman RB Bykov V Xue BG Xu H De Costa BR Jacobson AE Rice KC Kleinman JE Brady LS Interaction of opioid peptides and

other drugs with multiple kappa receptors in rat and human brain Evidence for species differences Peptides 1992 Sep Oct 13 5 977 87 PubMed PMID 1336192 Kinouchi K Pasternak GW Evidence for kappa 1 opioid receptor multiplicity in the guinea pig cerebellum Eur J Pharmacol 1991 Jun 19 207 2 135 41 PubMed PMID 1679014 Lahti RA Mickelson MM McCall JM Von Voigtlander PF 3H U 69593 a highly selective ligand for the opioid kappa receptor Eur J Pharmacol 1985 Feb 26 109 2 281 4 PubMed PMID 2986999 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Rothman RB Bykov V Xue BG Xu H De Costa BR Jacobson AE Rice KC Kleinman JE Brady LS Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain Evidence for species differences Peptides 1992 Sep Oct 13 5 977 87 PubMed PMID 1336192 Kinouchi K Pasternak GW Evidence for kappa 1 opioid receptor multiplicity in the guinea pig cerebellum Eur J Pharmacol 1991 Jun 19 207 2 135 41 PubMed PMID 1679014 Lahti RA Mickelson MM McCall JM Von Voigtlander PF 3H U 69593 a highly selective ligand for the opioid kappa receptor Eur J Pharmacol 1985 Feb 26 109 2 281 4 PubMed PMID 2986999 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Rothman RB Bykov V Xue BG Xu H De Costa BR Jacobson AE Rice KC Kleinman JE Brady LS Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain Evidence for species differences Peptides 1992 Sep Oct 13 5 977 87 PubMed PMID 1336192 Kinouchi K Pasternak GW Evidence for kappa 1 opioid receptor multiplicity in the guinea pig cerebellum Eur J Pharmacol 1991 Jun 19 207 2 135 41 PubMed PMID 1679014 Lahti RA Mickelson MM McCall JM Von Voigtlander PF 3H U 69593 a highly selective ligand for the opioid kappa receptor Eur J Pharmacol 1985 Feb 26 109 2 281 4 PubMed PMID 2986999 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Rothman RB Bykov V Xue BG Xu H De Costa BR Jacobson AE Rice KC Kleinman JE Brady LS Interaction of opioid peptides and other drugs with multiple kappa receptors in rat and human brain Evidence for species differences Peptides 1992 Sep Oct 13 5 977 87 PubMed PMID 1336192 Kinouchi K Pasternak GW Evidence for kappa 1 opioid receptor multiplicity in the guinea pig cerebellum Eur J Pharmacol 1991 Jun 19 207 2

135 41 PubMed PMID 1679014 Lahti RA Mickelson MM McCall JM Von Voigtlander PF 3H U 69593 a highly selective ligand for the opioid kappa receptor Eur J Pharmacol 1985 Feb 26 109 2 281 4 PubMed PMID 2986999 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_h5HT2A

Assay Title: Novascreen human unspecified 5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_h5HT2A was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_h5HT2A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR2A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_h5HT2A, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_h5HT2A assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_h5HT2A is a biochemical, single-readout assay that uses extracted gene-proteins from Human cortex in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4758.15
Neutral control median absolute deviation, by plate:	81.7283
Positive control well median response value, by plate:	1423.44
Positive control well median absolute deviation, by plate:	49.8598
Negative control well median, by plate:	98.6884573931389
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-34.93
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.03479178738798
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.2087507243279

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Leysen JE, Niemegeers CJ, Van Nueten JM, Laduron PM. [3H]Ketanserin (R 41468), a selective 3H-ligand for serotonin2 receptor binding sites. Binding properties, brain distribution, and functional role. Mol Pharmacol. 1982 Mar;21(2):301-14. PubMed PMID: 7099138.|Martin GR, Humphrey PP. Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. Neuropharmacology. 1994 Mar-Apr;33(3-4):261-73. Review. PubMed PMID: 7984266.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Leysen JE Niemegeers CJ Van Nueten JM Laduron PM 3H Ketanserin R 41468 a selective 3H ligand for serotonin2 receptor binding sites Binding properties brain distribution and functional role Mol Pharmacol 1982 Mar 21 2 301 14 PubMed PMID 7099138 Martin GR Humphrey PP Receptors for 5 hydroxytryptamine current perspectives on classification and nomenclature Neuropharmacology 1994 Mar Apr 33 3 4 261 73 Review PubMed PMID 7984266 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_h5HT5A

Assay Title: Novascreen human unspecified 5-hydroxytryptamine (serotonin) receptor 5A, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_h5HT5A was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_h5HT5A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR5A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_h5HT5A, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_h5HT5A assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_h5HT5A is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5809
Neutral control median absolute deviation, by plate:	355.824
Positive control well median response value, by plate:	208.5
Positive control well median absolute deviation, by plate:	14.826
Negative control well median, by plate:	117.170209028632
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.73
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.52
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.20885451353248
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.2531270811949

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Rees S, den Daas I, Foord S, Goodson S, Bull D, Kilpatrick G, Lee M. Cloning and characterisation of the human 5-HT5A serotonin receptor. FEBS Lett. 1994 Dec 5;355(3):242-6. PubMed PMID: 7988681.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Rees S den Daas I Foord S Goodson S Bull D Kilpatrick G Lee M Cloning and characterisation of the human 5 HT5A serotonin receptor FEBS Lett 1994 Dec 5 355 3 242 6 PubMed PMID 7988681 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_h5HT6

Assay Title: Novascreen human unspecified 5-hydroxytryptamine (serotonin) receptor 6, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_h5HT6 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_h5HT6, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR6. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_h5HT6, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_h5HT6 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_h5HT6 is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3495
Neutral control median absolute deviation, by plate:	204.599
Positive control well median response value, by plate:	312
Positive control well median absolute deviation, by plate:	34.8411
Negative control well median, by plate:	121.640287769784
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-13.84
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.07099779227296
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	36.4259867536378

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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References:

Monsma FJ Jr, Shen Y, Ward RP, Hamblin MW, Sibley DR. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol. 1993 Mar;43(3):320-7. PubMed PMID: 7680751.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Monsma FJ Jr Shen Y Ward RP Hamblin MW Sibley DR Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs Mol Pharmacol 1993 Mar 43 3 320 7 PubMed PMID 7680751 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_h5HT7

Assay Title: Novascreen human unspecified 5-hydroxytryptamine (serotonin) receptor 7, adenylate cyclase-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_h5HT7 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_h5HT7, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR7. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_h5HT7, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_h5HT7 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_h5HT7 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3023
Neutral control median absolute deviation, by plate:	204.599
Positive control well median response value, by plate:	341
Positive control well median absolute deviation, by plate:	37.065
Negative control well median, by plate:	111.752577319588
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.69
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-12.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.01418654281782
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.0851192569069

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Shen Y, Monsma FJ Jr, Metcalf MA, Jose PA, Hamblin MW, Sibley DR. Molecular cloning and expression of a 5-hydroxytryptamine7 serotonin receptor subtype. J Biol Chem. 1993 Aug 25;268(24):18200-4. PubMed PMID: 8394362.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Shen Y Monsma FJ Jr Metcalf MA Jose PA Hamblin MW Sibley DR Molecular cloning and expression of a 5 hydroxytryptamine7 serotonin receptor subtype J Biol Chem 1993 Aug 25 268 24 18200 4 PubMed PMID 8394362 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdoRA1

Assay Title: Novascreen human unspecified adenosine A1 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdoRA1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdoRA1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADORA1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_hAdoRA1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdoRA1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdoRA1 is a biochemical, single-readout assay that uses extracted gene-proteins from Human cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5325.5
Neutral control median absolute deviation, by plate:	293.555
Positive control well median response value, by plate:	526.5
Positive control well median absolute deviation, by plate:	26.6868
Negative control well median, by plate:	112.408357771261
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.76
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.83
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.8847922103881
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.3087532623286

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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Bruns RF, Fergus JH, Badger EW, Bristol JA, Santay LA, Hartman JD, Hays SJ, Huang CC. Binding of the A1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. Naunyn Schmiedebergs Arch Pharmacol. 1987 Jan;335(1):59-63. PubMed PMID: 3574492.|Ferkany, J. W., Valentine, H. L., Stone, G. A. and Williams, M. (1986), Adenosine A1 receptors in mammalian brain: Species differences in their interactions with agonists and antagonists. Drug Dev. Res., 9: 85â€“93. doi: 10.1002/ddr.430090202|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdoRA2a

Assay Title: Novascreen human unspecified adenosine A2a receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdoRA2a was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdoRA2a, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADORA2A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hAdoRA2a, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdoRA2a assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdoRA2a is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10251
Neutral control median absolute deviation, by plate:	597.488
Positive control well median response value, by plate:	650
Positive control well median absolute deviation, by plate:	34.0998
Negative control well median, by plate:	102.453700367515
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.79
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.37
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.91457259857584
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.487435591455

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Jarvis MF, Schulz R, Hutchison AJ, Do UH, Sills MA, Williams M. [3H]CGS 21680, a selective A2 adenosine receptor agonist directly labels A2 receptors in rat brain. J Pharmacol Exp Ther. 1989 Dec;251(3):888-93. PubMed PMID: 2600819.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Jarvis MF Schulz R Hutchison AJ Do UH Sills MA Williams M 3H CGS 21680 a selective A2 adenosine receptor agonist directly labels A2 receptors in rat brain J Pharmacol Exp Ther 1989 Dec 251 3 888 93 PubMed PMID 2600819 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdra2A

Assay Title: Novascreen human unspecified adrenoceptor alpha 2A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdra2A was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdra2A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADRA2A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hAdra2A, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdra2A assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdra2A is a biochemical, single-readout assay that uses extracted gene-proteins from HT29 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4558.93
Neutral control median absolute deviation, by plate:	135.673
Positive control well median response value, by plate:	366.865
Positive control well median absolute deviation, by plate:	14.3516
Negative control well median, by plate:	104.305903422142
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.96
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.43620196726539
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.6172118035923

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Pettibone DJ, Flagg SD, Totaro JA, Clineschmidt BV, Huff JR, Young SD, Chen R. [3H]L-657,743 (MK-912): a new, high affinity, selective radioligand for brain alpha 2-adrenoceptors. Life Sci. 1989;44(7):459-67. PubMed PMID: 2564617.|Bylund DB, Ray-Prenger C, Murphy TJ. Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. J Pharmacol Exp Ther. 1988 May;245(2):600-7. PubMed PMID: 2835476.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Pettibone DJ Flagg SD Totaro JA Clineschmidt BV Huff JR Young SD Chen R 3H L 657 743 MK 912 a new high affinity selective radioligand for brain alpha 2 adrenoceptors Life Sci 1989 44 7 459 67 PubMed PMID 2564617 Bylund DB Ray Prenger C Murphy TJ Alpha 2A and alpha 2B adrenergic receptor subtypes antagonist binding in tissues and cell lines containing only one subtype J Pharmacol Exp Ther 1988 May 245 2 600 7 PubMed PMID 2835476 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdra2C

Assay Title: Novascreen human unspecified adrenoceptor alpha 2C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdra2C was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdra2C, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADRA2C. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hAdra2C, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdra2C assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdra2C is a biochemical, single-readout assay that uses extracted gene-proteins from SF9 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7499
Neutral control median absolute deviation, by plate:	256.49
Positive control well median response value, by plate:	265.5
Positive control well median absolute deviation, by plate:	26.6868
Negative control well median, by plate:	114.981769970169
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.19
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.69672937859252
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.1803762715551

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdrb1

Assay Title: Novascreen human unspecified adrenoceptor beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdrb1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdrb1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADRB1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hAdrb1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdrb1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdrb1 is a biochemical, single-readout assay that uses extracted gene-proteins from Human neuroepithelioma cells in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3711
Neutral control median absolute deviation, by plate:	71.9061
Positive control well median response value, by plate:	494
Positive control well median absolute deviation, by plate:	40.0302
Negative control well median, by plate:	113.357282821685
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-34.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.56935036534774
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Minneman KP, Hegstrand LR, Molinoff PB. Simultaneous determination of beta-1 and beta-2-adrenergic receptors in tissues containing both receptor subtypes. Mol Pharmacol. 1979 Jul;16(1):34-46. PubMed PMID: 39246.|Kalaria RN, Andorn AC, Tabaton M, Whitehouse PJ, Harik SI, Unnerstall JR. Adrenergic receptors in aging and Alzheimer's disease: increased beta 2-receptors in prefrontal cortex and hippocampus. J Neurochem. 1989 Dec;53(6):1772-81. PubMed PMID: 2553864.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Minneman KP Hegstrand LR Molinoff PB Simultaneous determination of beta 1 and beta 2 adrenergic receptors in tissues containing both receptor subtypes Mol Pharmacol 1979 Jul 16 1 34 46 PubMed PMID 39246 Kalaria RN Andorn AC Tabaton M Whitehouse PJ Harik SI Unnerstall JR Adrenergic receptors in aging and Alzheimer s disease increased beta 2 receptors in prefrontal cortex and hippocampus J Neurochem 1989 Dec 53 6 1772 81 PubMed PMID 2553864 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdrb2

Assay Title: Novascreen human unspecified adrenoceptor beta 2, surface assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdrb2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdrb2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADRB2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hAdrb2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdrb2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdrb2 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5034
Neutral control median absolute deviation, by plate:	348.411
Positive control well median response value, by plate:	673.5
Positive control well median absolute deviation, by plate:	43.7367
Negative control well median, by plate:	105.030880082347
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.99688487472681
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Minneman KP, Hegstrand LR, Molinoff PB. Simultaneous determination of beta-1 and beta-2-adrenergic receptors in tissues containing both receptor subtypes. Mol Pharmacol. 1979 Jul;16(1):34-46. PubMed PMID: 39246.|Kalaria RN, Andorn AC, Tabaton M, Whitehouse PJ, Harik SI, Unnerstall JR. Adrenergic receptors in aging and Alzheimer's disease: increased beta 2-receptors in prefrontal cortex and hippocampus. J Neurochem. 1989 Dec;53(6):1772-81. PubMed PMID: 2553864.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Minneman KP Hegstrand LR Molinoff PB Simultaneous determination of beta 1 and beta 2 adrenergic receptors in tissues containing both receptor subtypes Mol Pharmacol 1979 Jul 16 1 34 46 PubMed PMID 39246 Kalaria RN Andorn AC Tabaton M Whitehouse PJ Harik SI Unnerstall JR Adrenergic receptors in aging and Alzheimer s disease increased beta 2 receptors in prefrontal cortex and hippocampus J Neurochem 1989 Dec 53 6 1772 81 PubMed PMID 2553864 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAdrb3

Assay Title: Novascreen human unspecified adrenoceptor beta 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAdrb3 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAdrb3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene ADRB3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hAdrb3, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAdrb3 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAdrb3 is a biochemical, single-readout assay that uses extracted gene-proteins from SK-N-MC pretreated with isoproteronol and 8-bromo-cAMP in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	26392.7
Neutral control median absolute deviation, by plate:	1287.19
Positive control well median response value, by plate:	11473.7
Positive control well median absolute deviation, by plate:	408.605
Negative control well median, by plate:	119.846398305085
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.61
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-10
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.42
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.08032380840602
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.4819428504361

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Chaudhry A, Granneman JG. Influence of cell type upon the desensitization of the beta 3-adrenergic receptor. J Pharmacol Exp Ther. 1994 Dec;271(3):1253-8. PubMed PMID: 7996434.|Curran PK, Fishman PH. Endogenous beta 3- but not beta 1-adrenergic receptors are resistant to agonist-mediated regulation in human SK-N-MC neurotumor cells. Cell Signal. 1996 Aug;8(5):355-64. PubMed PMID: 8911684.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hAT1

Assay Title: Novascreen human unspecified angiotensin II receptor, type 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hAT1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hAT1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene AGTR1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_hAT1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hAT1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hAT1 is a biochemical, single-readout assay that uses extracted gene-proteins from KAN-TS in a tissue-based cell-free assay.Measurements were taken 3 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2985
Neutral control median absolute deviation, by plate:	106.747
Positive control well median response value, by plate:	429
Positive control well median absolute deviation, by plate:	32.6172
Negative control well median, by plate:	103.52422907489
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.61614463361047
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.6968678016628

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Bennett JP Jr, Snyder SH. Angiotensin II binding to mammalian brain membranes. J Biol Chem. 1976 Dec 10;251(23):7423-30. PubMed PMID: 187587.|Wiest SA, Rampersaud A, Zimmerman K, Steinberg MI. Characterization of distinct angiotensin II binding sites in rat adrenal gland and bovine cerebellum using selective nonpeptide antagonists. J Cardiovasc Pharmacol. 1991 Feb;17(2):177-84. PubMed PMID: 1709220.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Bennett JP Jr Snyder SH Angiotensin II binding to mammalian brain membranes J Biol Chem 1976 Dec 10 251 23 7423 30 PubMed PMID 187587 Wiest SA Rampersaud A Zimmerman K Steinberg MI Characterization of distinct angiotensin II binding sites in rat adrenal gland and bovine cerebellum using selective nonpeptide antagonists J Cardiovasc Pharmacol 1991 Feb 17 2 177 84 PubMed PMID 1709220 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hC5a

Assay Title: Novascreen human unspecified complement component 5a receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hC5a was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hC5a, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene C5AR1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hC5a, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hC5a assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hC5a is a biochemical, single-readout assay that uses extracted gene-proteins from U937 in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	10681.5
Neutral control median absolute deviation, by plate:	484.81
Positive control well median response value, by plate:	819.5
Positive control well median absolute deviation, by plate:	63.0105
Negative control well median, by plate:	98.5791456095444
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-31.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.49376392500225
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Zimmerli W, Reber AM, Dahinden CA. The role of formylpeptide receptors, C5a receptors, and cytosolic-free calcium in neutrophil priming. J Infect Dis. 1990 Feb;161(2):242-9. PubMed PMID: 2153739.|Johnson RJ, Chenoweth DE. Synthesis of a new photoreactive C5a analog that permits identification of the ligand binding component of the granulocyte C5a receptor. Biochem Biophys Res Commun. 1987 Nov 13;148(3):1330-7. PubMed PMID: 3689398.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Zimmerli W Reber AM Dahinden CA The role of formylpeptide receptors C5a receptors and cytosolic free calcium in neutrophil priming J Infect Dis 1990 Feb 161 2 242 9 PubMed PMID 2153739 Johnson RJ Chenoweth DE Synthesis of a new photoreactive C5a analog that permits identification of the ligand binding component of the granulocyte C5a receptor Biochem Biophys Res Commun 1987 Nov 13 148 3 1330 7 PubMed PMID 3689398 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hDRD1

Assay Title: Novascreen human unspecified dopamine receptor D1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hDRD1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hDRD1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene DRD1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hDRD1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hDRD1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hDRD1 is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5684
Neutral control median absolute deviation, by plate:	137.14
Positive control well median response value, by plate:	108
Positive control well median absolute deviation, by plate:	14.826
Negative control well median, by plate:	108.779576587796
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-31.29
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.24915747148213
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Billard W, Ruperto V, Crosby G, Iorio LC, Barnett A. Characterization of the binding of 3H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. Life Sci. 1984 Oct 29;35(18):1885-93. PubMed PMID: 6387355.|Jarvie KR, Tiberi M, Silvia C, Gingrich JA, Caron MG. Molecular cloning, stable expression and desensitization of the human dopamine D1b/D5 receptor. J Recept Res. 1993;13(1-4):573-90. PubMed PMID: 8450505.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hDRD2s

Assay Title: Novascreen human unspecified dopamine receptor D2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hDRD2s was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hDRD2s, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene DRD2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hDRD2s, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hDRD2s assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hDRD2s is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3792
Neutral control median absolute deviation, by plate:	124.538
Positive control well median response value, by plate:	115
Positive control well median absolute deviation, by plate:	26.6868
Negative control well median, by plate:	114.035333707235
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.3168142686669
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.9008856120014

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gundlach AL, Largent BL, Snyder SH. 125I-Spiperone: a novel ligand for D2 dopamine receptors. Life Sci. 1984 Nov 5;35(19):1981-8. PubMed PMID: 6149442.|Jarvie KR, Tiberi M, Silvia C, Gingrich JA, Caron MG. Molecular cloning, stable expression and desensitization of the human dopamine D1b/D5 receptor. J Recept Res. 1993;13(1-4):573-90. PubMed PMID: 8450505.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hDRD4.4

Assay Title: Novascreen human unspecified dopamine receptor D4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hDRD4.4 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hDRD4.4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene DRD4. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hDRD4.4, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hDRD4.4 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hDRD4.4 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3766
Neutral control median absolute deviation, by plate:	259.455
Positive control well median response value, by plate:	297
Positive control well median absolute deviation, by plate:	19.2738
Negative control well median, by plate:	105.710730088496
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.75
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-13.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.3173606214533
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. Multiple dopamine D4 receptor variants in the human population. Nature. 1992 Jul 9;358(6382):149-52. PubMed PMID: 1319557.|Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature. 1991 Apr 18;350(6319):610-4. PubMed PMID: 1840645.|Seeman P, Van Tol HH. Dopamine D4 receptors bind inactive (+)-aporphines, suggesting neuroleptic role. Sulpiride not stereoselective. Eur J Pharmacol. 1993 Mar 16;233(1):173-4. PubMed PMID: 8097160.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Van Tol HH Wu CM Guan HC Ohara K Bunzow JR Civelli O Kennedy J Seeman P Niznik HB Jovanovic V Multiple dopamine D4 receptor variants in the human population Nature 1992 Jul 9 358 6382 149 52 PubMed PMID 1319557 Van Tol HH Bunzow JR Guan HC Sunahara RK Seeman P Niznik HB Civelli O Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine Nature 1991 Apr 18 350 6319 610 4 PubMed PMID 1840645 Seeman P Van Tol HH Dopamine D4 receptors bind inactive aporphines suggesting neuroleptic role Sulpiride not stereoselective Eur J Pharmacol 1993 Mar 16 233 1 173 4 PubMed PMID 8097160 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Van Tol HH Wu CM Guan HC Ohara K Bunzow JR Civelli O Kennedy J Seeman P Niznik HB Jovanovic V Multiple dopamine D4 receptor variants in the human population Nature 1992 Jul 9 358 6382 149 52

PubMed PMID 1319557 Van Tol HH Bunzow JR Guan HC Sunahara RK Seeman P Niznik HB Civelli O Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine Nature 1991 Apr 18 350 6319 610 4 PubMed PMID 1840645 Seeman P Van Tol HH Dopamine D4 receptors bind inactive aporphines suggesting neuroleptic role Sulpiride not stereoselective Eur J Pharmacol 1993 Mar 16 233 1 173 4 PubMed PMID 8097160 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hETA

Assay Title: Novascreen human unspecified endothelin receptor type A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hETA was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hETA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene EDNRA. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hETA, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hETA assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hETA is a biochemical, single-readout assay that uses extracted gene-proteins from Human neuroblastoma cells (SH-SY5Y) in a cell-free assay.Measurements were taken 3 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4481.5
Neutral control median absolute deviation, by plate:	178.653
Positive control well median response value, by plate:	1215
Positive control well median absolute deviation, by plate:	42.2541
Negative control well median, by plate:	118.493930197269
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-19
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-20.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.6263307125508
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.7579842753048

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Bolger GT, Liard F, Krogsrud R, Thibeault D, Jaramillo J. Tissue specificity of endothelin binding sites. J Cardiovasc Pharmacol. 1990 Sep;16(3):367-75. PubMed PMID: 1700206.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hETB

Assay Title: Novascreen human unspecified endothelin receptor type B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hETB was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hETB, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene EDNRB. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hETB, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hETB assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hETB is a biochemical, single-readout assay that uses extracted gene-proteins from Human astrocytoma cells in a cell-free assay.Measurements were taken 4 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4799
Neutral control median absolute deviation, by plate:	106.747
Positive control well median response value, by plate:	1176
Positive control well median absolute deviation, by plate:	34.0998
Negative control well median, by plate:	97.3020527859237
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-17.84
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.25
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.61905302176655
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.7143181305993

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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References:

Wu-Wong JR, Chiou WJ, Magnuson SR, Opgenorth TJ. Endothelin receptor in human astrocytoma U373MG cells: binding, dissociation, receptor internalization. J Pharmacol Exp Ther. 1995 Jul;274(1):499-507. PubMed PMID: 7616437.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Wu Wong JR Chiou WJ Magnuson SR Opgenorth TJ Endothelin receptor in human astrocytoma U373MG cells binding dissociation receptor internalization J Pharmacol Exp Ther 1995 Jul 274 1 499 507 PubMed PMID 7616437 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hH1

Assay Title: Novascreen human unspecified histamine receptor H1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hH1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hH1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HRH1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hH1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hH1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hH1 is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7962
Neutral control median absolute deviation, by plate:	177.912
Positive control well median response value, by plate:	184
Positive control well median absolute deviation, by plate:	28.1694
Negative control well median, by plate:	101.251309509952
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.02
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.16547675294364
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Chang RS, Tran VT, Snyder SH. Heterogeneity of histamine H1-receptors: species variations in [3H]mepyramine binding of brain membranes. J Neurochem. 1979 Jun;32(6):1653-63. PubMed PMID: 448359.|Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, Palacios JM. Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. Brain Res. 1990 Sep 3;526(2):322-7. PubMed PMID: 1979518.|Haaksma EE, Leurs R, Timmerman H. Histamine receptors: subclasses and specific ligands. Pharmacol Ther. 1990;47(1):73-104. Review. PubMed PMID: 2195558.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hLTB4_BLT1

Assay Title: Novascreen human unspecified leukotriene B4 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hLTB4_BLT1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hLTB4_BLT1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene LTB4R. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hLTB4_BLT1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hLTB4_BLT1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hLTB4_BLT1 is a biochemical, single-readout assay that uses extracted gene-proteins from Human neutrophils in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3545.72
Neutral control median absolute deviation, by plate:	103.07
Positive control well median response value, by plate:	357.89
Positive control well median absolute deviation, by plate:	20.6823
Negative control well median, by plate:	110.654914772012
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.14
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.57292770427424
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.4375662256454

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T. A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. Nature. 1997 Jun 5;387(6633):620-4. PubMed PMID: 9177352.|Kato K, Yokomizo T, Izumi T, Shimizu T. Cell-specific transcriptional regulation of human leukotriene B(4) receptor gene. J Exp Med. 2000 Aug 7;192(3):413-20. PubMed PMID: 10934229; PubMed Central PMCID: PMC2193224.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Yokomizo T Izumi T Chang K Takuwa Y Shimizu T A G protein coupled receptor for leukotriene B4 that mediates chemotaxis Nature 1997 Jun 5 387 6633 620 4 PubMed PMID 9177352 Kato K Yokomizo T Izumi T Shimizu T Cell specific transcriptional regulation of human leukotriene B 4 receptor gene J Exp Med 2000 Aug 7 192 3 413 20 PubMed PMID 10934229 PubMed Central PMCID PMC2193224 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hM1

Assay Title: Novascreen human unspecified cholinergic receptor, muscarinic 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hM1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hM1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene CHRM1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hM1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hM1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hM1 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4979
Neutral control median absolute deviation, by plate:	249.077
Positive control well median response value, by plate:	203
Positive control well median absolute deviation, by plate:	40.0302
Negative control well median, by plate:	107.874794069193
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.73131427166235
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.3878856299741

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Buckley NJ, Bonner TI, Buckley CM, Brann MR. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. Mol Pharmacol. 1989 Apr;35(4):469-76. PubMed PMID: 2704370.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hM2

Assay Title: Novascreen human unspecified cholinergic receptor, muscarinic 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hM2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hM2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene CHRM2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hM2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hM2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hM2 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5958
Neutral control median absolute deviation, by plate:	280.211
Positive control well median response value, by plate:	177
Positive control well median absolute deviation, by plate:	25.2042
Negative control well median, by plate:	108.120225838592
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.79
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.97
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.77642516122107
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.6585509673264

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Buckley NJ, Bonner TI, Buckley CM, Brann MR. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. Mol Pharmacol. 1989 Apr;35(4):469-76. PubMed PMID: 2704370.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hM3

Assay Title: Novascreen human unspecified cholinergic receptor, muscarinic 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hM3 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hM3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene CHRM3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hM3, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hM3 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hM3 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4310
Neutral control median absolute deviation, by plate:	174.947
Positive control well median response value, by plate:	120
Positive control well median absolute deviation, by plate:	26.6868
Negative control well median, by plate:	105.533141210375
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-19
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.41782329169728
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.5069397501837

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hM4

Assay Title: Novascreen human unspecified cholinergic receptor, muscarinic 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hM4 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hM4, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene CHRM4. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hM4, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hM4 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hM4 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1568
Neutral control median absolute deviation, by plate:	62.2692
Positive control well median response value, by plate:	127
Positive control well median absolute deviation, by plate:	53.3736
Negative control well median, by plate:	114.055700609225
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.73
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.42655726877634
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.559343612658

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

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Buckley NJ, Bonner TI, Buckley CM, Brann MR. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. Mol Pharmacol. 1989 Apr;35(4):469-76. PubMed PMID: 2704370.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hM5

Assay Title: Novascreen human unspecified cholinergic receptor, muscarinic 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hM5 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hM5, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene CHRM5. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hM5, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hM5 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hM5 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4168
Neutral control median absolute deviation, by plate:	140.847
Positive control well median response value, by plate:	221
Positive control well median absolute deviation, by plate:	45.9606
Negative control well median, by plate:	110.438237418733
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.75
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.39
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.04635741164073
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.2781444698444

Chemical Library Scope and Limitations:

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25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hNK2

Assay Title: Novascreen human unspecified tachykinin receptor 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hNK2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hNK2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene TACR2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hNK2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hNK2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hNK2 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 4 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	12913
Neutral control median absolute deviation, by plate:	590.075
Positive control well median response value, by plate:	683
Positive control well median absolute deviation, by plate:	37.065
Negative control well median, by plate:	114.36929789766
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-26.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.87246328134604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Regoli D, Nantel F. Pharmacology of neurokinin receptors. Biopolymers. 1991 May;31(6):777-83. Review. PubMed PMID: 1718474.|Burcher E, Buck SH, Lovenberg W, O'Donohue TL. Characterization and autoradiographic localization of multiple tachykinin binding sites in gastrointestinal tract and bladder. J Pharmacol Exp Ther. 1986 Mar;236(3):819-31. PubMed PMID: 2419545.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Regoli D Nantel F Pharmacology of neurokinin receptors Biopolymers 1991 May 31 6 777 83 Review PubMed PMID 1718474 Burcher E Buck SH Lovenberg W O Donohue TL Characterization and autoradiographic localization of multiple tachykinin binding sites in gastrointestinal tract and bladder J Pharmacol Exp Ther 1986 Mar 236 3 819 31 PubMed PMID 2419545 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hNPY1

Assay Title: Novascreen human unspecified neuropeptide Y receptor Y1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hNPY1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hNPY1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NPY1R. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hNPY1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hNPY1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hNPY1 is a biochemical, single-readout assay that uses extracted gene-proteins from SK-N-MC in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2793
Neutral control median absolute deviation, by plate:	106.747
Positive control well median response value, by plate:	700
Positive control well median absolute deviation, by plate:	56.3388
Negative control well median, by plate:	123.282674772036
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.76
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.78155011923047
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.6893007153828

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

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NCI, Normalized Cell Index

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SULT, Sulfotransferases

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hNPY2

Assay Title: Novascreen human unspecified neuropeptide Y receptor Y2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hNPY2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hNPY2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NPY2R. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hNPY2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hNPY2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hNPY2 is a biochemical, single-readout assay that uses extracted gene-proteins from SK-N-MC in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4419.5
Neutral control median absolute deviation, by plate:	107.488
Positive control well median response value, by plate:	618
Positive control well median absolute deviation, by plate:	32.6172
Negative control well median, by plate:	101.597968936679
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-33
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hNTS

Assay Title: Novascreen human unspecified neurotensin receptor 1 (high affinity) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hNTS was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hNTS, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NTSR1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hNTS, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hNTS assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hNTS is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2973.5
Neutral control median absolute deviation, by plate:	207.564
Positive control well median response value, by plate:	624.5
Positive control well median absolute deviation, by plate:	61.5279
Negative control well median, by plate:	100.345238095238
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.64
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-10
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.55
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.65095733511501
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.9057440106901

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Goedert M, Pittaway K, Williams BJ, Emson PC. Specific binding of tritiated neurotensin to rat brain membranes: characterization and regional distribution. Brain Res. 1984 Jun 18;304(1):71-81. PubMed PMID: 6744039.|Gully D, Canton M, Boigegrain R, Jeanjean F, Molimard JC, Poncelet M, Gueudet C, Heaulme M, Leyris R, Brouard A, et al. Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of the neurotensin receptor. Proc Natl Acad Sci U S A. 1993 Jan 1;90(1):65-9. PubMed PMID: 8380498; PubMed Central PMCID: PMC45600.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hOpiate_D1

Assay Title: Novascreen human unspecified opioid receptor, delta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hOpiate_D1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hOpiate_D1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene OPRD1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hOpiate_D1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hOpiate_D1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hOpiate_D1 is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3813
Neutral control median absolute deviation, by plate:	197.186
Positive control well median response value, by plate:	224
Positive control well median absolute deviation, by plate:	35.5824
Negative control well median, by plate:	110.216144684605
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.36
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.70323783533576
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.2194270120146

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Malatynska E, Wang Y, Knapp RJ, Santoro G, Li X, Waite S, Roeske WR, Yamamura HI. Human delta opioid receptor: a stable cell line for functional studies of opioids. Neuroreport. 1995 Mar 7;6(4):613-6. PubMed PMID: 7605911.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Malatynska E Wang Y Knapp RJ Santoro G Li X Waite S Roeske WR Yamamura HI Human delta opioid receptor a stable cell line for functional studies of opioids Neuroreport 1995 Mar 7 6 4 613 6 PubMed PMID 7605911 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hOpiate_mu

Assay Title: Novascreen human unspecified opioid receptor, mu 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hOpiate_mu was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hOpiate_mu, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene OPRM1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hOpiate_mu, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hOpiate_mu assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hOpiate_mu is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 2.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6036.5
Neutral control median absolute deviation, by plate:	311.346
Positive control well median response value, by plate:	187.5
Positive control well median absolute deviation, by plate:	20.7564
Negative control well median, by plate:	109.111445783133
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.81
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.26308018031029
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.5784810818617

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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DMSO, Dimethyl Sulfoxide

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GST, Glutathione S-Transferase

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hORL1

Assay Title: Novascreen human unspecified opiate receptor-like 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hORL1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hORL1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene OPRL1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hORL1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hORL1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hORL1 is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1570.69
Neutral control median absolute deviation, by plate:	45.2934
Positive control well median response value, by plate:	207.14
Positive control well median absolute deviation, by plate:	10.4523
Negative control well median, by plate:	108.499529702243
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.14461879121983
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.867712747319

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, Civelli O. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science. 1995 Nov 3;270(5237):792-4. PubMed PMID: 7481766.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Reinscheid RK Nothacker HP Bourson A Ardati A Henningsen RA Bunzow JR Grandy DK Langen H Monsma FJ Jr Civelli O Orphanin FQ a neuropeptide that activates an opioidlike G protein coupled receptor Science 1995 Nov 3 270 5237 792 4 PubMed PMID 7481766 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hPY2

Assay Title: Novascreen human unspecified purinergic receptor P2Y, G-protein coupled, 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hPY2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hPY2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene P2RY1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hPY2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hPY2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hPY2 is a biochemical, single-readout assay that uses extracted gene-proteins from U937 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	12275
Neutral control median absolute deviation, by plate:	387.218
Positive control well median response value, by plate:	2987.36
Positive control well median absolute deviation, by plate:	122.233
Negative control well median, by plate:	80.9287881917193
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.58
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.67878456834974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	34.0727074100984

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Cooper CL, Morris AJ, Harden TK. Guanine nucleotide-sensitive interaction of a radiolabeled agonist with a phospholipase C-linked P2y-purinergic receptor. J Biol Chem. 1989 Apr 15;264(11):6202-6. PubMed PMID: 2495280.|Levin RM, Jacoby R, Wein AJ. High-affinity, divalent ion-specific binding of 3H-ATP to homogenate derived from rabbit urinary bladder. Comparison with divalent-ion ATPase activity. Mol Pharmacol. 1983 Jan;23(1):1-7. PubMed PMID: 6306420.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hTXA2

Assay Title: Novascreen human unspecified thromboxane A2 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hTXA2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hTXA2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene TBXA2R. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hTXA2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hTXA2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hTXA2 is a biochemical, single-readout assay that uses extracted gene-proteins from Human platelets in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4427.86
Neutral control median absolute deviation, by plate:	220.455
Positive control well median response value, by plate:	2093.98
Positive control well median absolute deviation, by plate:	100.476
Negative control well median, by plate:	150.343464825363
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.66
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.49275455712066
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.956527342724

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hedberg A, Hall SE, Ogletree ML, Harris DN, Liu EC. Characterization of [5,6-3H]SQ 29,548 as a high affinity radioligand, binding to thromboxane A2/prostaglandin H2-receptors in human platelets. J Pharmacol Exp Ther. 1988 Jun;245(3):786-92. PubMed PMID: 2968449.|Armstrong RA, Jones RL, Wilson NH. Ligand binding to thromboxane receptors on human platelets: correlation with biological activity. Br J Pharmacol. 1983 Aug;79(4):953-64. PubMed PMID: 6317122; PubMed Central PMCID: PMC2044935.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hV1A

Assay Title: Novascreen human unspecified arginine vasopressin receptor 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hV1A was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hV1A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene AVPR1A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hV1A, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hV1A assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hV1A is a biochemical, single-readout assay that uses extracted gene-proteins from Human platelets in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13461
Neutral control median absolute deviation, by plate:	432.178
Positive control well median response value, by plate:	1256
Positive control well median absolute deviation, by plate:	54.8562
Negative control well median, by plate:	101.410424879544
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-32.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.16317225818328
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Vittet D, Rondot A, Cantau B, Launay JM, Chevillard C. Nature and properties of human platelet vasopressin receptors. Biochem J. 1986 Feb 1;233(3):631-6. PubMed PMID: 3010940; PubMed Central PMCID: PMC1153078.|Dorsa DM, Petracca FM, Baskin DG, Cornett LE. Localization and characterization of vasopressin-binding sites in the amygdala of the rat brain. J Neurosci. 1984 Jul;4(7):1764-70. PubMed PMID: 6330316.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Vittet D Rondot A Cantau B Launay JM Chevillard C Nature and properties of human platelet vasopressin receptors Biochem J 1986 Feb 1 233 3 631 6 PubMed PMID 3010940 PubMed Central PMCID PMC1153078 Dorsa DM Petracca FM Baskin DG Cornett LE Localization and characterization of vasopressin binding sites in the amygdala of the rat brain J Neurosci 1984 Jul 4 7 1764 70 PubMed PMID 6330316 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_mCCKAPeripheral

Assay Title: Novascreen mouse unspecified cholecystokinin A receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_mCCKAPeripheral was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_mCCKAPeripheral, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cckar. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_mCCKAPeripheral, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_mCCKAPeripheral assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_mCCKAPeripheral is a biochemical, single-readout assay that uses extracted gene-proteins from Mouse pancreatic membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6229.5
Neutral control median absolute deviation, by plate:	262.42
Positive control well median response value, by plate:	391.5
Positive control well median absolute deviation, by plate:	18.5325
Negative control well median, by plate:	101.089588377724
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.17593805429475
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Wennogle LP, Steel DJ, Petrack B. Characterization of central cholecystokinin receptors using a radioiodinated octapeptide probe. Life Sci. 1985 Apr 15;36(15):1485-92. PubMed PMID: 2984502.|Innis RB, Snyder SH. Distinct cholecystokinin receptors in brain and pancreas. Proc Natl Acad Sci U S A. 1980 Nov;77(11):6917-21. PubMed PMID: 6256771; PubMed Central PMCID: PMC350402.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Wennogle LP Steel DJ Petrack B Characterization of central cholecystokinin receptors using a radioiodinated octapeptide probe Life Sci 1985 Apr 15 36 15 1485 92 PubMed PMID 2984502 Innis RB Snyder SH Distinct cholecystokinin receptors in brain and pancreas Proc Natl Acad Sci U S A 1980 Nov 77 11 6917 21 PubMed PMID 6256771 PubMed Central PMCID PMC350402 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_mCKKBCentral

Assay Title: Novascreen mouse unspecified cholecystokinin B receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_mCKKBCentral was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_mCKKBCentral, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cckbr. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_mCKKBCentral, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_mCKKBCentral assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_mCKKBCentral is a biochemical, single-readout assay that uses extracted gene-proteins from Mouse forebrain membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3903.5
Neutral control median absolute deviation, by plate:	95.6277
Positive control well median response value, by plate:	865
Positive control well median absolute deviation, by plate:	22.9803
Negative control well median, by plate:	95.6926903274795
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-39.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.33628048780488
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.0176829268293

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_p5HT2C

Assay Title: Novascreen pig unspecified 5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_p5HT2C was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_p5HT2C, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR2C. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_p5HT2C, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_p5HT2C assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_p5HT2C is a biochemical, single-readout assay that uses extracted gene-proteins from Pig choroid plexus membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4748.92
Neutral control median absolute deviation, by plate:	152.745
Positive control well median response value, by plate:	1354.15
Positive control well median absolute deviation, by plate:	54.5523
Negative control well median, by plate:	113.572235619488
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.28
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.98045777577634
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.882746654658

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hoyer D, Engel G, Kalkman HO. Molecular pharmacology of 5-HT1 and 5-HT2 recognition sites in rat and pig brain membranes: radioligand binding studies with [3H]5-HT, [3H]8-OH-DPAT, (-)[125I]iodocyanopindolol, [3H]mesulergine and [3H]ketanserin. Eur J Pharmacol. 1985 Nov 26;118(1-2):13-23. PubMed PMID: 2935410.|Pazos A, Hoyer D, Palacios JM. The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. Eur J Pharmacol. 1984 Nov 27;106(3):539-46. PubMed PMID: 6519175.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Hoyer D Engel G Kalkman HO Molecular pharmacology of 5 HT1 and 5 HT2 recognition sites in rat and pig brain membranes radioligand binding studies with 3H 5 HT 3H 8 OH DPAT 125I iodocyanopindolol 3H mesulergine and 3H ketanserin Eur J Pharmacol 1985 Nov 26 118 1 2 13 23 PubMed PMID 2935410 Pazos A Hoyer D Palacios JM The binding of serotonergic ligands to the porcine choroid plexus characterization of a new type of serotonin recognition site Eur J Pharmacol 1984 Nov 27 106 3 539 46 PubMed PMID 6519175 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_r5HT_NonSelective

Assay Title: Novascreen rat unspecified 5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_r5HT_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_r5HT_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Htr1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_r5HT_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_r5HT_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_r5HT_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17332.7
Neutral control median absolute deviation, by plate:	378.182
Positive control well median response value, by plate:	3609.69
Positive control well median absolute deviation, by plate:	142.241
Negative control well median, by plate:	102.350946767703
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.63050050995029
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.7830030597017

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Peroutka SJ, Snyder SH. Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiroperidol. Mol Pharmacol. 1979 Nov;16(3):687-99. PubMed PMID: 530254.|Peroutka SJ, Snyder SH. Two distinct serotonin receptors: regional variations in receptor binding in mammalian brain. Brain Res. 1981 Mar 16;208(2):339-47. PubMed PMID: 7214150.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Peroutka SJ Snyder SH Multiple serotonin receptors differential binding of 3H 5 hydroxytryptamine 3H lysergic acid diethylamide and 3H spiroperidol Mol Pharmacol 1979 Nov 16 3 687 99 PubMed PMID 530254 Peroutka SJ Snyder SH Two distinct serotonin receptors regional variations in receptor binding in mammalian brain Brain Res 1981 Mar 16 208 2 339 47 PubMed PMID 7214150 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_r5HT1_NonSelective

Assay Title: Novascreen rat unspecified 5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_r5HT1_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_r5HT1_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Htr1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_r5HT1_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_r5HT1_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_r5HT1_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5813.55
Neutral control median absolute deviation, by plate:	183.946
Positive control well median response value, by plate:	2423.6
Positive control well median absolute deviation, by plate:	78.5778
Negative control well median, by plate:	118.105115014381
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.73
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.94
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.43
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.59477239412067
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.568634364724

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rabPAF

Assay Title: Novascreen rabbit unspecified platelet-activating factor receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rabPAF was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rabPAF, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene PTAFR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rabPAF, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rabPAF assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rabPAF is a biochemical, single-readout assay that uses extracted gene-proteins from Rabbit platelets in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7978.48
Neutral control median absolute deviation, by plate:	192.278
Positive control well median response value, by plate:	2875.24
Positive control well median absolute deviation, by plate:	86.3021
Negative control well median, by plate:	98.9318262028293
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-26.84
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.37
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

model

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.96861289220113
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.8116773532068

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hwang SB, Lam MH, Biftu T, Beattie TR, Shen TY. Trans-2,5-Bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran. An orally active specific and competitive receptor antagonist of platelet activating factor. J Biol Chem. 1985 Dec 15;260(29):15639-45. PubMed PMID: 2999126.|Hwang SB, Lee CS, Cheah MJ, Shen TY. Specific receptor sites for 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine (platelet activating factor) on rabbit platelet and guinea pig smooth muscle membranes. Biochemistry. 1983 Sep 27;22(20):4756-63. PubMed PMID: 6313047.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Hwang SB Lam MH Biftu T Beattie TR Shen TY Trans 2 5 Bis 3 4 5 trimethoxyphenyl tetrahydrofuran An orally active specific and competitive receptor antagonist of platelet activating factor J Biol Chem 1985 Dec 15 260 29 15639 45 PubMed PMID 2999126 Hwang SB Lee CS Cheah MJ Shen TY Specific receptor sites for 1 O alkyl 2 O acetyl sn glycero 3 phosphocholine platelet activating factor on rabbit platelet and guinea pig smooth muscle membranes Biochemistry 1983 Sep 27 22 20 4756 63 PubMed PMID 6313047 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Hwang SB Lam MH Biftu T Beattie TR Shen TY Trans 2 5 Bis 3 4 5 trimethoxyphenyl tetrahydrofuran An orally active specific and competitive receptor antagonist of platelet activating factor J Biol Chem 1985 Dec 15 260 29 15639 45 PubMed PMID 2999126 Hwang SB Lee CS Cheah MJ Shen TY Specific receptor sites for 1 O alkyl 2 O acetyl sn glycero 3 phosphocholine platelet activating factor on rabbit

platelet and guinea pig smooth muscle membranes Biochemistry 1983 Sep 27 22 20 4756 63 PubMed PMID 6313047 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Hwang SB Lam MH Biftu T Beattie TR Shen TY Trans 2 5 Bis 3 4 5 trimethoxyphenyl tetrahydrofuran An orally active specific and competitive receptor antagonist of platelet activating factor J Biol Chem 1985 Dec 15 260 29 15639 45 PubMed PMID 2999126 Hwang SB Lee CS Cheah MJ Shen TY Specific receptor sites for 1 O alkyl 2 O acetyl sn glycero 3 phosphocholine platelet activating factor on rabbit platelet and guinea pig smooth muscle membranes Biochemistry 1983 Sep 27 22 20 4756 63 PubMed PMID 6313047 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rAdra1_NonSelective

Assay Title: Novascreen rat unspecified adrenoceptor alpha 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rAdra1_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rAdra1_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adra1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rAdra1_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rAdra1_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rAdra1_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2499.86
Neutral control median absolute deviation, by plate:	48.9629
Positive control well median response value, by plate:	328.95
Positive control well median absolute deviation, by plate:	14.6407
Negative control well median, by plate:	112.714381366876
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-35
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-42.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.76916947223126
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.6150168333876

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Reader TA, BriÃ¨re R, Grondin L. Alpha-1 and alpha-2 adrenoceptor binding in cerebral cortex: competition studies with [3H]prazosin and [3H]idazoxan. J Neural Transm. 1987;68(1-2):79-95. PubMed PMID: 2879884.|Timmermans PB, Ali FK, Kwa HY, Schoop AM, Slothorst-Grisdijk FP, van Zwieten PA. Identical antagonist selectivity of central and peripheral alpha 1-adrenoceptors. Mol Pharmacol. 1981 Sep;20(2):295-301. PubMed PMID: 6117785.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Reader TA Bri re R Grondin L Alpha 1 and alpha 2 adrenoceptor binding in cerebral cortex competition studies with 3H prazosin and 3H idazoxan J Neural Transm 1987 68 1 2 79 95 PubMed PMID 2879884 Timmermans PB Ali FK Kwa HY Schoop AM Slothorst Grisdijk FP van Zwieten PA Identical antagonist selectivity of central and peripheral alpha 1 adrenoceptors Mol Pharmacol 1981 Sep 20 2 295 301 PubMed PMID 6117785 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hORL1

Assay Title: Novascreen human unspecified opiate receptor-like 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hORL1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hORL1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene OPRL1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hORL1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hORL1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hORL1 is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1570.69
Neutral control median absolute deviation, by plate:	45.2934
Positive control well median response value, by plate:	207.14
Positive control well median absolute deviation, by plate:	10.4523
Negative control well median, by plate:	108.499529702243
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.14461879121983
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.867712747319

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, Civelli O. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science. 1995 Nov 3;270(5237):792-4. PubMed PMID: 7481766.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Reinscheid RK Nothacker HP Bourson A Ardati A Henningsen RA Bunzow JR Grandy DK Langen H Monsma FJ Jr Civelli O Orphanin FQ a neuropeptide that activates an opioidlike G protein coupled receptor Science 1995 Nov 3 270 5237 792 4 PubMed PMID 7481766 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hPY2

Assay Title: Novascreen human unspecified purinergic receptor P2Y, G-protein coupled, 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hPY2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hPY2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene P2RY1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hPY2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hPY2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hPY2 is a biochemical, single-readout assay that uses extracted gene-proteins from U937 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	12275
Neutral control median absolute deviation, by plate:	387.218
Positive control well median response value, by plate:	2987.36
Positive control well median absolute deviation, by plate:	122.233
Negative control well median, by plate:	80.9287881917193
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.58
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.67878456834974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	34.0727074100984

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Cooper CL, Morris AJ, Harden TK. Guanine nucleotide-sensitive interaction of a radiolabeled agonist with a phospholipase C-linked P2y-purinergic receptor. J Biol Chem. 1989 Apr 15;264(11):6202-6. PubMed PMID: 2495280.|Levin RM, Jacoby R, Wein AJ. High-affinity, divalent ion-specific binding of 3H-ATP to homogenate derived from rabbit urinary bladder. Comparison with divalent-ion ATPase activity. Mol Pharmacol. 1983 Jan;23(1):1-7. PubMed PMID: 6306420.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hTXA2

Assay Title: Novascreen human unspecified thromboxane A2 receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hTXA2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hTXA2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene TBXA2R. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hTXA2, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hTXA2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hTXA2 is a biochemical, single-readout assay that uses extracted gene-proteins from Human platelets in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4427.86
Neutral control median absolute deviation, by plate:	220.455
Positive control well median response value, by plate:	2093.98
Positive control well median absolute deviation, by plate:	100.476
Negative control well median, by plate:	150.343464825363
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.66
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.49275455712066
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.956527342724

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hedberg A, Hall SE, Ogletree ML, Harris DN, Liu EC. Characterization of [5,6-3H]SQ 29,548 as a high affinity radioligand, binding to thromboxane A2/prostaglandin H2-receptors in human platelets. J Pharmacol Exp Ther. 1988 Jun;245(3):786-92. PubMed PMID: 2968449.|Armstrong RA, Jones RL, Wilson NH. Ligand binding to thromboxane receptors on human platelets: correlation with biological activity. Br J Pharmacol. 1983 Aug;79(4):953-64. PubMed PMID: 6317122; PubMed Central PMCID: PMC2044935.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_hV1A

Assay Title: Novascreen human unspecified arginine vasopressin receptor 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_hV1A was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_hV1A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene AVPR1A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_GPCR_hV1A, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_hV1A assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_hV1A is a biochemical, single-readout assay that uses extracted gene-proteins from Human platelets in a cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	13461
Neutral control median absolute deviation, by plate:	432.178
Positive control well median response value, by plate:	1256
Positive control well median absolute deviation, by plate:	54.8562
Negative control well median, by plate:	101.410424879544
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-32.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.16317225818328
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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References:

Vittet D, Rondot A, Cantau B, Launay JM, Chevillard C. Nature and properties of human platelet vasopressin receptors. Biochem J. 1986 Feb 1;233(3):631-6. PubMed PMID: 3010940; PubMed Central PMCID: PMC1153078.|Dorsa DM, Petracca FM, Baskin DG, Cornett LE. Localization and characterization of vasopressin-binding sites in the amygdala of the rat brain. J Neurosci. 1984 Jul;4(7):1764-70. PubMed PMID: 6330316.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Vittet D Rondot A Cantau B Launay JM Chevillard C Nature and properties of human platelet vasopressin receptors Biochem J 1986 Feb 1 233 3 631 6 PubMed PMID 3010940 PubMed Central PMCID PMC1153078 Dorsa DM Petracca FM Baskin DG Cornett LE Localization and characterization of vasopressin binding sites in the amygdala of the rat brain J Neurosci 1984 Jul 4 7 1764 70 PubMed PMID 6330316 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_mCCKAPeripheral

Assay Title: Novascreen mouse unspecified cholecystokinin A receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_mCCKAPeripheral was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_mCCKAPeripheral, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cckar. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_mCCKAPeripheral, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_mCCKAPeripheral assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_mCCKAPeripheral is a biochemical, single-readout assay that uses extracted gene-proteins from Mouse pancreatic membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6229.5
Neutral control median absolute deviation, by plate:	262.42
Positive control well median response value, by plate:	391.5
Positive control well median absolute deviation, by plate:	18.5325
Negative control well median, by plate:	101.089588377724
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.17593805429475
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Wennogle LP, Steel DJ, Petrack B. Characterization of central cholecystokinin receptors using a radioiodinated octapeptide probe. Life Sci. 1985 Apr 15;36(15):1485-92. PubMed PMID: 2984502.|Innis RB, Snyder SH. Distinct cholecystokinin receptors in brain and pancreas. Proc Natl Acad Sci U S A. 1980 Nov;77(11):6917-21. PubMed PMID: 6256771; PubMed Central PMCID: PMC350402.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Wennogle LP Steel DJ Petrack B Characterization of central cholecystokinin receptors using a radioiodinated octapeptide probe Life Sci 1985 Apr 15 36 15 1485 92 PubMed PMID 2984502 Innis RB Snyder SH Distinct cholecystokinin receptors in brain and pancreas Proc Natl Acad Sci U S A 1980 Nov 77 11 6917 21 PubMed PMID 6256771 PubMed Central PMCID PMC350402 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_mCKKBCentral

Assay Title: Novascreen mouse unspecified cholecystokinin B receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_mCKKBCentral was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_mCKKBCentral, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cckbr. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_mCKKBCentral, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_mCKKBCentral assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_mCKKBCentral is a biochemical, single-readout assay that uses extracted gene-proteins from Mouse forebrain membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3903.5
Neutral control median absolute deviation, by plate:	95.6277
Positive control well median response value, by plate:	865
Positive control well median absolute deviation, by plate:	22.9803
Negative control well median, by plate:	95.6926903274795
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-39.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.33628048780488
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.0176829268293

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_p5HT2C

Assay Title: Novascreen pig unspecified 5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_p5HT2C was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_p5HT2C, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR2C. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_p5HT2C, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_p5HT2C assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_p5HT2C is a biochemical, single-readout assay that uses extracted gene-proteins from Pig choroid plexus membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4748.92
Neutral control median absolute deviation, by plate:	152.745
Positive control well median response value, by plate:	1354.15
Positive control well median absolute deviation, by plate:	54.5523
Negative control well median, by plate:	113.572235619488
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.28
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

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3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.98045777577634
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.882746654658

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hoyer D, Engel G, Kalkman HO. Molecular pharmacology of 5-HT1 and 5-HT2 recognition sites in rat and pig brain membranes: radioligand binding studies with [3H]5-HT, [3H]8-OH-DPAT, (-)[125I]iodocyanopindolol, [3H]mesulergine and [3H]ketanserin. Eur J Pharmacol. 1985 Nov 26;118(1-2):13-23. PubMed PMID: 2935410.|Pazos A, Hoyer D, Palacios JM. The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. Eur J Pharmacol. 1984 Nov 27;106(3):539-46. PubMed PMID: 6519175.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Hoyer D Engel G Kalkman HO Molecular pharmacology of 5 HT1 and 5 HT2 recognition sites in rat and pig brain membranes radioligand binding studies with 3H 5 HT 3H 8 OH DPAT 125I iodocyanopindolol 3H mesulergine and 3H ketanserin Eur J Pharmacol 1985 Nov 26 118 1 2 13 23 PubMed PMID 2935410 Pazos A Hoyer D Palacios JM The binding of serotonergic ligands to the porcine choroid plexus characterization of a new type of serotonin recognition site Eur J Pharmacol 1984 Nov 27 106 3 539 46 PubMed PMID 6519175 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_r5HT_NonSelective

Assay Title: Novascreen rat unspecified 5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_r5HT_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_r5HT_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Htr1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_r5HT_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_r5HT_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_r5HT_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	17332.7
Neutral control median absolute deviation, by plate:	378.182
Positive control well median response value, by plate:	3609.69
Positive control well median absolute deviation, by plate:	142.241
Negative control well median, by plate:	102.350946767703
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.63050050995029
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.7830030597017

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Peroutka SJ, Snyder SH. Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiroperidol. Mol Pharmacol. 1979 Nov;16(3):687-99. PubMed PMID: 530254.|Peroutka SJ, Snyder SH. Two distinct serotonin receptors: regional variations in receptor binding in mammalian brain. Brain Res. 1981 Mar 16;208(2):339-47. PubMed PMID: 7214150.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Peroutka SJ Snyder SH Multiple serotonin receptors differential binding of 3H 5 hydroxytryptamine 3H lysergic acid diethylamide and 3H spiroperidol Mol Pharmacol 1979 Nov 16 3 687 99 PubMed PMID 530254 Peroutka SJ Snyder SH Two distinct serotonin receptors regional variations in receptor binding in mammalian brain Brain Res 1981 Mar 16 208 2 339 47 PubMed PMID 7214150 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_r5HT1_NonSelective

Assay Title: Novascreen rat unspecified 5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_r5HT1_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_r5HT1_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Htr1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_r5HT1_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_r5HT1_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_r5HT1_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5813.55
Neutral control median absolute deviation, by plate:	183.946
Positive control well median response value, by plate:	2423.6
Positive control well median absolute deviation, by plate:	78.5778
Negative control well median, by plate:	118.105115014381
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.73
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.94
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.43
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.59477239412067
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.568634364724

Chemical Library Scope and Limitations:

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rabPAF

Assay Title: Novascreen rabbit unspecified platelet-activating factor receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rabPAF was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rabPAF, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene PTAFR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rabPAF, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rabPAF assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rabPAF is a biochemical, single-readout assay that uses extracted gene-proteins from Rabbit platelets in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7978.48
Neutral control median absolute deviation, by plate:	192.278
Positive control well median response value, by plate:	2875.24
Positive control well median absolute deviation, by plate:	86.3021
Negative control well median, by plate:	98.9318262028293
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-26.84
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.37
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

model

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.96861289220113
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.8116773532068

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hwang SB, Lam MH, Biftu T, Beattie TR, Shen TY. Trans-2,5-Bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran. An orally active specific and competitive receptor antagonist of platelet activating factor. J Biol Chem. 1985 Dec 15;260(29):15639-45. PubMed PMID: 2999126.|Hwang SB, Lee CS, Cheah MJ, Shen TY. Specific receptor sites for 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine (platelet activating factor) on rabbit platelet and guinea pig smooth muscle membranes. Biochemistry. 1983 Sep 27;22(20):4756-63. PubMed PMID: 6313047.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Hwang SB Lam MH Biftu T Beattie TR Shen TY Trans 2 5 Bis 3 4 5 trimethoxyphenyl tetrahydrofuran An orally active specific and competitive receptor antagonist of platelet activating factor J Biol Chem 1985 Dec 15 260 29 15639 45 PubMed PMID 2999126 Hwang SB Lee CS Cheah MJ Shen TY Specific receptor sites for 1 O alkyl 2 O acetyl sn glycero 3 phosphocholine platelet activating factor on rabbit platelet and guinea pig smooth muscle membranes Biochemistry 1983 Sep 27 22 20 4756 63 PubMed PMID 6313047 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Hwang SB Lam MH Biftu T Beattie TR Shen TY Trans 2 5 Bis 3 4 5 trimethoxyphenyl tetrahydrofuran An orally active specific and competitive receptor antagonist of platelet activating factor J Biol Chem 1985 Dec 15 260 29 15639 45 PubMed PMID 2999126 Hwang SB Lee CS Cheah MJ Shen TY Specific receptor sites for 1 O alkyl 2 O acetyl sn glycero 3 phosphocholine platelet activating factor on rabbit

platelet and guinea pig smooth muscle membranes Biochemistry 1983 Sep 27 22 20 4756 63 PubMed PMID 6313047 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Hwang SB Lam MH Biftu T Beattie TR Shen TY Trans 2 5 Bis 3 4 5 trimethoxyphenyl tetrahydrofuran An orally active specific and competitive receptor antagonist of platelet activating factor J Biol Chem 1985 Dec 15 260 29 15639 45 PubMed PMID 2999126 Hwang SB Lee CS Cheah MJ Shen TY Specific receptor sites for 1 O alkyl 2 O acetyl sn glycero 3 phosphocholine platelet activating factor on rabbit platelet and guinea pig smooth muscle membranes Biochemistry 1983 Sep 27 22 20 4756 63 PubMed PMID 6313047 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rAdra1_NonSelective

Assay Title: Novascreen rat unspecified adrenoceptor alpha 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rAdra1_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rAdra1_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adra1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rAdra1_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rAdra1_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rAdra1_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2499.86
Neutral control median absolute deviation, by plate:	48.9629
Positive control well median response value, by plate:	328.95
Positive control well median absolute deviation, by plate:	14.6407
Negative control well median, by plate:	112.714381366876
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-35
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-42.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.76916947223126
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.6150168333876

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Reader TA, BriÃ¨re R, Grondin L. Alpha-1 and alpha-2 adrenoceptor binding in cerebral cortex: competition studies with [3H]prazosin and [3H]idazoxan. J Neural Transm. 1987;68(1-2):79-95. PubMed PMID: 2879884.|Timmermans PB, Ali FK, Kwa HY, Schoop AM, Slothorst-Grisdijk FP, van Zwieten PA. Identical antagonist selectivity of central and peripheral alpha 1-adrenoceptors. Mol Pharmacol. 1981 Sep;20(2):295-301. PubMed PMID: 6117785.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Reader TA Bri re R Grondin L Alpha 1 and alpha 2 adrenoceptor binding in cerebral cortex competition studies with 3H prazosin and 3H idazoxan J Neural Transm 1987 68 1 2 79 95 PubMed PMID 2879884 Timmermans PB Ali FK Kwa HY Schoop AM Slothorst Grisdijk FP van Zwieten PA Identical antagonist selectivity of central and peripheral alpha 1 adrenoceptors Mol Pharmacol 1981 Sep 20 2 295 301 PubMed PMID 6117785 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rAdra1A

Assay Title: Novascreen rat unspecified adrenoceptor alpha 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rAdra1A was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rAdra1A, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adra1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rAdra1A, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rAdra1A assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rAdra1A is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes (pretreated with chlorethyl clonidine-CEC) in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1800.75
Neutral control median absolute deviation, by plate:	78.007
Positive control well median response value, by plate:	589.73
Positive control well median absolute deviation, by plate:	31.3125
Negative control well median, by plate:	123.05467754871
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-26.09
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.59548173286397
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.5728903971838

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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Author of Revisions:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rAdra1B

Assay Title: Novascreen rat unspecified adrenoceptor alpha 1B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rAdra1B was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rAdra1B, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adra1b. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rAdra1B, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rAdra1B assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rAdra1B is a biochemical, single-readout assay that uses extracted gene-proteins from Rat liver membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1788.15
Neutral control median absolute deviation, by plate:	68.5999
Positive control well median response value, by plate:	377.42
Positive control well median absolute deviation, by plate:	45.2267
Negative control well median, by plate:	110.138189858319
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.76
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.17
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.60108389013527
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.6065033408116

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Hanft G, Gross G. Subclassification of alpha 1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists. Br J Pharmacol. 1989 Jul;97(3):691-700. PubMed PMID: 2569345; PubMed Central PMCID: PMC1854578.|Minneman KP, Han C, Abel PW. Comparison of alpha 1-adrenergic receptor subtypes distinguished by chlorethylclonidine and WB 4101. Mol Pharmacol. 1988 May;33(5):509-14. PubMed PMID: 2835650.|Gross G, Hanft G, Rugevics C. 5-Methyl-urapidil discriminates between subtypes of the alpha 1-adrenoceptor. Eur J Pharmacol. 1988 Jul 7;151(2):333-5. PubMed PMID: 2901974.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Hanft G Gross G Subclassification of alpha 1 adrenoceptor recognition sites by urapidil derivatives and other selective antagonists Br J Pharmacol 1989 Jul 97 3 691 700 PubMed PMID 2569345 PubMed Central PMCID PMC1854578 Minneman KP Han C Abel PW Comparison of alpha 1 adrenergic receptor subtypes distinguished by chlorethylclonidine and WB 4101 Mol Pharmacol 1988 May 33 5 509 14 PubMed PMID 2835650 Gross G

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rAdra2_NonSelective

Assay Title: Novascreen rat unspecified adrenoceptor alpha 2A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rAdra2_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rAdra2_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adra2a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rAdra2_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rAdra2_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rAdra2_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1.25 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2197.67
Neutral control median absolute deviation, by plate:	44.478
Positive control well median response value, by plate:	247.84
Positive control well median absolute deviation, by plate:	19.6889
Negative control well median, by plate:	106.797193916969
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-44.44
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.83974222709743
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.0384533625846

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Reader TA, BriÃ¨re R, Grondin L. Alpha-1 and alpha-2 adrenoceptor binding in cerebral cortex: competition studies with [3H]prazosin and [3H]idazoxan. J Neural Transm. 1987;68(1-2):79-95. PubMed PMID: 2879884.|O'Rourke MF, Blaxall HS, Iversen LJ, Bylund DB. Characterization of [3H]RX821002 binding to alpha-2 adrenergic receptor subtypes. J Pharmacol Exp Ther. 1994 Mar;268(3):1362-7. PubMed PMID: 7908054.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Reader TA Bri re R Grondin L Alpha 1 and alpha 2 adrenoceptor binding in cerebral cortex competition studies with 3H prazosin and 3H idazoxan J Neural Transm 1987 68 1 2 79 95 PubMed PMID 2879884 O Rourke MF Blaxall HS Iversen LJ Bylund DB Characterization of 3H RX821002 binding to alpha 2 adrenergic receptor subtypes J Pharmacol Exp Ther 1994 Mar 268 3 1362 7 PubMed PMID 7908054 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rAdrb_NonSelective

Assay Title: Novascreen rat unspecified adrenoceptor beta 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rAdrb_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rAdrb_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adrb1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rAdrb_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rAdrb_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rAdrb_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	11451.4
Neutral control median absolute deviation, by plate:	290.797
Positive control well median response value, by plate:	3665.38
Positive control well median absolute deviation, by plate:	172.53
Negative control well median, by plate:	123.033959719372
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.64
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.35
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.02735110341008
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Arango V, Ernsberger P, Reis DJ, Mann JJ. Demonstration of high- and low-affinity beta-adrenergic receptors in slide-mounted sections of rat and human brain. Brain Res. 1990 May 14;516(1):113-21. PubMed PMID: 2163719.|Riva MA, Creese I. Reevaluation of the regulation of beta-adrenergic receptor binding by desipramine treatment. Mol Pharmacol. 1989 Jul;36(1):211-8. PubMed PMID: 2546051.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rCRF

Assay Title: Novascreen rat unspecified corticotropin releasing hormone receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rCRF was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rCRF, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Crhr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "secretin receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rCRF, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rCRF assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rCRF is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7340.5
Neutral control median absolute deviation, by plate:	270.426
Positive control well median response value, by plate:	2824.35
Positive control well median absolute deviation, by plate:	96.8138
Negative control well median, by plate:	84.6136674020381
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.68
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-17.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.29345668341154
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.7607401004692

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

De Souza EB, Insel TR, Perrin MH, Rivier J, Vale WW, Kuhar MJ. Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. J Neurosci. 1985 Dec;5(12):3189-203. PubMed PMID: 3001239.|De Souza EB. Corticotropin-releasing factor receptors in the rat central nervous system: characterization and regional distribution. J Neurosci. 1987 Jan;7(1):88-100. PubMed PMID: 3027279.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rGABBR

Assay Title: Novascreen rat unspecified gamma-aminobutyric acid (GABA) B receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rGABBR was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rGABBR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Gabbr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "metabotropic glutamate receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rGABBR, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rGABBR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rGABBR is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4689
Neutral control median absolute deviation, by plate:	386.959
Positive control well median response value, by plate:	699
Positive control well median absolute deviation, by plate:	45.2193
Negative control well median, by plate:	101.103074141049
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.59
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-9.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.09

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.7263968906667
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.3583813440002

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Scherer RW, Ferkany JW, Enna SJ. Evidence for pharmacologically distinct subsets of GABAB receptors. Brain Res Bull. 1988 Sep;21(3):439-43. PubMed PMID: 2850843.|Bittiger, H., Reymann, N., Froestl, W., & Mickel, S. J. (1992). H-CGP54626: a potent antagonist radioligand for GABA B receptors. Pharmacol. Commun, 2, 23.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Scherer RW Ferkany JW Enna SJ Evidence for pharmacologically distinct subsets of GABAB receptors Brain Res Bull 1988 Sep 21 3 439 43 PubMed PMID 2850843 Bittiger H Reymann N Froestl W Mickel S J 1992 H CGP54626 a potent antagonist radioligand for GABA B receptors Pharmacol Commun 2 23 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rGalanin

Assay Title: Novascreen rat unspecified galanin receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rGalanin was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rGalanin, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Galr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rGalanin, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rGalanin assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rGalanin is a biochemical, single-readout assay that uses extracted gene-proteins from Rat brain membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3770
Neutral control median absolute deviation, by plate:	74.13
Positive control well median response value, by plate:	1009
Positive control well median absolute deviation, by plate:	48.9258
Negative control well median, by plate:	99.3265007320644
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-47.42
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	9.26625
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	55.5975

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

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Skofitsch G, Sills MA, Jacobowitz DM. Autoradiographic distribution of 125I-galanin binding sites in the rat central nervous system. Peptides. 1986 Nov-Dec;7(6):1029-42. PubMed PMID: 2436195.|Servin AL, Amiranoff B, Rouyer-Fessard C, Tatemoto K, Laburthe M. Identification and molecular characterization of galanin receptor sites in rat brain. Biochem Biophys Res Commun. 1987 Apr 14;144(1):298-306. PubMed PMID: 2437911.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Skofitsch G Sills MA Jacobowitz DM Autoradiographic distribution of 125I galanin binding sites in the rat central nervous system Peptides 1986 Nov Dec 7 6 1029 42 PubMed PMID 2436195 Servin AL Amiranoff B Rouyer Fessard C Tatemoto K Laburthe M Identification and molecular characterization of galanin receptor sites in rat brain Biochem Biophys Res Commun 1987 Apr 14 144 1 298 306 PubMed PMID 2437911 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rGHB

Assay Title: Novascreen rat unspecified tetraspanin 17 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rGHB was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rGHB, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Tspan17. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "metabotropic glutamate receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rGHB, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rGHB assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rGHB is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7350.44
Neutral control median absolute deviation, by plate:	170.64
Positive control well median response value, by plate:	1328.71
Positive control well median absolute deviation, by plate:	44.567
Negative control well median, by plate:	96.6600578000941
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.27
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.2441462348676
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Bourguignon JJ, Schmitt M, Didier B. Design and structure-activity relationship analysis of ligands of gamma-hydroxybutyric acid receptors. Alcohol. 2000 Apr;20(3):227-36. Review. PubMed PMID: 10869864.|Carter LP, Wu H, Chen W, Matthews MM, Mehta AK, Hernandez RJ, Thomson JA, Ticku MK, Coop A, Koek W, France CP. Novel gamma-hydroxybutyric acid (GHB) analogs share some, but not all, of the behavioral effects of GHB and GABAB receptor agonists. J Pharmacol Exp Ther. 2005 Jun;313(3):1314-23. Epub 2005 Mar 15. PubMed PMID: 15769868.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rH3

Assay Title: Novascreen rat unspecified histamine receptor H3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rH3 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rH3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Hrh3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rH3, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rH3 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rH3 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2230.14
Neutral control median absolute deviation, by plate:	58.9556
Positive control well median response value, by plate:	245.925
Positive control well median absolute deviation, by plate:	12.3426
Negative control well median, by plate:	109.289464431506
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-33
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-34.68
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.68295701770653
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

West RE Jr, Zweig A, Shih NY, Siegel MI, Egan RW, Clark MA. Identification of two H3-histamine receptor subtypes. Mol Pharmacol. 1990 Nov;38(5):610-3. PubMed PMID: 2172771.|Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, Schunack W, Schwartz JC. Highly potent and selective ligands for histamine H3-receptors. Nature. 1987 May 14-20;327(6118):117-23. PubMed PMID: 3033516.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|West RE Jr Zweig A Shih NY Siegel MI Egan RW Clark MA Identification of two H3 histamine receptor subtypes Mol Pharmacol 1990 Nov 38 5 610 3 PubMed PMID 2172771 Arrang JM Garbarg M Lancelot JC Lecomte JM Pollard H Robba M Schunack W Schwartz JC Highly potent and selective ligands for histamine H3 receptors Nature 1987 May 14 20 327 6118 117 23 PubMed PMID 3033516 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rmAdra2B

Assay Title: Novascreen rat unspecified adrenoceptor alpha 2B assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rmAdra2B was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rmAdra2B, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Adra2b. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rmAdra2B, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rmAdra2B assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rmAdra2B is a biochemical, single-readout assay that uses extracted gene-proteins from neuroglioma/blastoma hybrid cells in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4532.57
Neutral control median absolute deviation, by plate:	97.9109
Positive control well median response value, by plate:	433.45
Positive control well median absolute deviation, by plate:	23.1286
Negative control well median, by plate:	109.79774501301
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.21
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.26504791219465
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.5902874731679

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Pettibone DJ, Flagg SD, Totaro JA, Clineschmidt BV, Huff JR, Young SD, Chen R. [3H]L-657,743 (MK-912): a new, high affinity, selective radioligand for brain alpha 2-adrenoceptors. Life Sci. 1989;44(7):459-67. PubMed PMID: 2564617.|Bylund DB, Ray-Prenger C, Murphy TJ. Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. J Pharmacol Exp Ther. 1988 May;245(2):600-7. PubMed PMID: 2835476.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Pettibone DJ Flagg SD Totaro JA Clineschmidt BV Huff JR Young SD Chen R 3H L 657 743 MK 912 a new high affinity selective radioligand for brain alpha 2 adrenoceptors Life Sci 1989 44 7 459 67 PubMed PMID 2564617 Bylund DB Ray Prenger C Murphy TJ Alpha 2A and alpha 2B adrenergic receptor subtypes antagonist binding in tissues and cell lines containing only one subtype J Pharmacol Exp Ther 1988 May 245 2 600 7 PubMed PMID 2835476 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rmMGluR1

Assay Title: Novascreen rat unspecified glutamate receptor, metabotropic 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rmMGluR1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rmMGluR1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Grm1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "metabotropic glutamate receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rmMGluR1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rmMGluR1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rmMGluR1 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cerebellum in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6479
Neutral control median absolute deviation, by plate:	360.272
Positive control well median response value, by plate:	761
Positive control well median absolute deviation, by plate:	32.6172
Negative control well median, by plate:	113.988988988989
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.76
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.52
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.6624974974975
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.974984984985

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gasparini F, Andres H, Flor PJ, Heinrich M, Inderbitzin W, LingenhÃ¶hl K, MÃ¼ller H, Munk VC, Omilusik K, Stierlin C, Stoehr N, Vranesic I, Kuhn R. [(3)H]-M-MPEP, a potent, subtype-selective radioligand for the metabotropic glutamate receptor subtype 5. Bioorg Med Chem Lett. 2002 Feb 11;12(3):407-9. PubMed PMID: 11814808.|Ohashi H, Maruyama T, Higashi-Matsumoto H, Nomoto T, Nishimura S, Takeuchi Y. A novel binding assay for metabotropic glutamate receptors using [3H] L-quisqualic acid and recombinant receptors. Z Naturforsch C. 2002 Mar-Apr;57(3-4):348-55. PubMed PMID: 12064739.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rmMGluR5

Assay Title: Novascreen rat unspecified glutamate receptor, metabotropic 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rmMGluR5 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rmMGluR5, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Grm5. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "metabotropic glutamate receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rmMGluR5, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rmMGluR5 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rmMGluR5 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat whole brain in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	25460.6
Neutral control median absolute deviation, by plate:	618.541
Positive control well median response value, by plate:	5727.51
Positive control well median absolute deviation, by plate:	155.614
Negative control well median, by plate:	96.3263475755658
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.81
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.3
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.6352455594958
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rNK1

Assay Title: Novascreen rat unspecified tachykinin receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rNK1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rNK1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Tacr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rNK1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rNK1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rNK1 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat submaxillary gland membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5181.27
Neutral control median absolute deviation, by plate:	102.359
Positive control well median response value, by plate:	1531.56
Positive control well median absolute deviation, by plate:	30.4526
Negative control well median, by plate:	117.516412222149
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-32
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-38.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.33
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.05042045510251
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Regoli D, Nantel F. Pharmacology of neurokinin receptors. Biopolymers. 1991 May;31(6):777-83. Review. PubMed PMID: 1718474.|Bahouth SW, Musacchio JM. Specific binding of [3H]substance P to the rat submaxillary gland. The effects of ions and guanine nucleotides. J Pharmacol Exp Ther. 1985 Aug;234(2):326-36. PubMed PMID: 2410593.|McLean S, Ganong A, Seymour PA, Snider RM, Desai MC, Rosen T, Bryce DK, Longo KP, Reynolds LS, Robinson G, et al. Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. J Pharmacol Exp Ther. 1993 Oct;267(1):472-9. PubMed PMID: 7693914.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Regoli D Nantel F Pharmacology of neurokinin receptors Biopolymers 1991 May 31 6 777 83 Review PubMed PMID 1718474 Bahouth SW Musacchio JM Specific binding of 3H substance P to the rat submaxillary gland The effects of ions and guanine nucleotides J Pharmacol Exp Ther 1985 Aug 234 2 326 36 PubMed PMID 2410593 McLean S Ganong A Seymour PA Snider RM Desai MC Rosen T Bryce DK Longo KP Reynolds LS Robinson G et al Pharmacology of CP 99 994 a nonpeptide antagonist of the tachykinin neurokinin 1 receptor J Pharmacol Exp Ther 1993 Oct 267 1 472 9 PubMed PMID 7693914 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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FBS, Fetal Bovine Serum

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rNK3

Assay Title: Novascreen rat unspecified tachykinin receptor 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rNK3 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rNK3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Tacr3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rNK3, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rNK3 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rNK3 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2115.5
Neutral control median absolute deviation, by plate:	55.0045
Positive control well median response value, by plate:	795.4
Positive control well median absolute deviation, by plate:	35.2859
Negative control well median, by plate:	100.096718480138
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.72
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-20.83
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.35
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.26980736175446
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.6188441705268

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Regoli D, Nantel F. Pharmacology of neurokinin receptors. Biopolymers. 1991 May;31(6):777-83. Review. PubMed PMID: 1718474.|Mussap CJ, Burcher E. [125I]-Bolton-Hunter scyliorhinin II: a novel, selective radioligand for the tachykinin NK3 receptor in rat brain. Peptides. 1990 Jul-Aug;11(4):827-36. PubMed PMID: 2172946.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Regoli D Nantel F Pharmacology of neurokinin receptors Biopolymers 1991 May 31 6 777 83 Review PubMed PMID 1718474 Mussap CJ Burcher E 125I Bolton Hunter scyliorhinin II a novel selective radioligand for the tachykinin NK3 receptor in rat brain Peptides 1990 Jul Aug 11 4 827 36 PubMed PMID 2172946 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rNTS

Assay Title: Novascreen rat unspecified neurotensin receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rNTS was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rNTS, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Ntsr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rNTS, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rNTS assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rNTS is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3982.09
Neutral control median absolute deviation, by plate:	52.2172
Positive control well median response value, by plate:	895.2
Positive control well median absolute deviation, by plate:	36.042
Negative control well median, by plate:	96.0758129582644
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-30
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-53.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.22
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.10383891537175
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Goedert M, Pittaway K, Williams BJ, Emson PC. Specific binding of tritiated neurotensin to rat brain membranes: characterization and regional distribution. Brain Res. 1984 Jun 18;304(1):71-81. PubMed PMID: 6744039.|Gully D, Canton M, Boigegrain R, Jeanjean F, Molimard JC, Poncelet M, Gueudet C, Heaulme M, Leyris R, Brouard A, et al. Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of the neurotensin receptor. Proc Natl Acad Sci U S A. 1993 Jan 1;90(1):65-9. PubMed PMID: 8380498; PubMed Central PMCID: PMC45600.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rOpiate_NonSelective

Assay Title: Novascreen rat unspecified opioid receptor, mu 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rOpiate_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rOpiate_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Oprm1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rOpiate_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rOpiate_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rOpiate_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2563.28
Neutral control median absolute deviation, by plate:	74.9899
Positive control well median response value, by plate:	1031.02
Positive control well median absolute deviation, by plate:	30.9789
Negative control well median, by plate:	133.675435297533
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.79
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.41
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.62157936352171
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.7294761811303

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

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4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Kuhar MJ, Pert CB, Snyder SH. Regional distribution of opiate receptor binding in monkey and human brain. Nature. 1973 Oct 26;245(5426):447-50. PubMed PMID: 4127185.|Pert C and Snyder SH. Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium. Mol. Pharmacol. 19: 868-879 (1974)|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Kuhar MJ Pert CB Snyder SH Regional distribution of opiate receptor binding in monkey and human brain Nature 1973 Oct 26 245 5426 447 50 PubMed PMID 4127185 Pert C and Snyder SH Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium Mol Pharmacol 19 868 879 1974 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rOpiate_NonSelectiveNa

Assay Title: Novascreen rat unspecified opioid receptor, mu 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rOpiate_NonSelectiveNa was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rOpiate_NonSelectiveNa, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Oprm1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rOpiate_NonSelectiveNa, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rOpiate_NonSelectiveNa assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rOpiate_NonSelectiveNa is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3485.18
Neutral control median absolute deviation, by plate:	73.7742
Positive control well median response value, by plate:	1098.42
Positive control well median absolute deviation, by plate:	26.5163
Negative control well median, by plate:	113.818488648662
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.3
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.7260013867527
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.3560083205162

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Kuhar MJ, Pert CB, Snyder SH. Regional distribution of opiate receptor binding in monkey and human brain. Nature. 1973 Oct 26;245(5426):447-50. PubMed PMID: 4127185.|Pert C and Snyder SH. Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium. Mol. Pharmacol. 19: 868-879 (1974)|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Kuhar MJ Pert CB Snyder SH Regional distribution of opiate receptor binding in monkey and human brain Nature 1973 Oct 26 245 5426 447 50 PubMed PMID 4127185 Pert C and Snyder SH Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium Mol Pharmacol 19 868 879 1974 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rOXT

Assay Title: Novascreen rat unspecified oxytocin receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rOXT was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rOXT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Oxtr. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rOXT, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rOXT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rOXT is a biochemical, single-readout assay that uses extracted gene-proteins from Rat uterine membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2258.54
Neutral control median absolute deviation, by plate:	104.041
Positive control well median response value, by plate:	824.915
Positive control well median absolute deviation, by plate:	18.7845
Negative control well median, by plate:	109.639874119921
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.73
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.4
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.02899952210411
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Pettibone DJ, Woyden CJ, Totaro JA. Identification of functional oxytocin receptors in lactating rat mammary gland in vitro. Eur J Pharmacol. 1990 Apr 25;188(4-5):235-41. PubMed PMID: 2163875.|Fuchs AR, Behrens O, Helmer H, Liu CH, Barros CM, Fields MJ. Oxytocin and vasopressin receptors in bovine endometrium and myometrium during the estrous cycle and early pregnancy. Endocrinology. 1990 Aug;127(2):629-36. PubMed PMID: 2164915.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Pettibone DJ Woyden CJ Totaro JA Identification of functional oxytocin receptors in lactating rat mammary gland in vitro Eur J Pharmacol 1990 Apr 25 188 4 5 235 41 PubMed PMID 2163875 Fuchs AR Behrens O Helmer H Liu CH Barros CM Fields MJ Oxytocin and vasopressin receptors in bovine endometrium and myometrium during the estrous cycle and early pregnancy Endocrinology 1990 Aug 127 2 629 36 PubMed PMID 2164915 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rSST

Assay Title: Novascreen rat unspecified somatostatin receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rSST was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rSST, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Sstr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rSST, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rSST assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rSST is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	12014
Neutral control median absolute deviation, by plate:	530.771
Positive control well median response value, by plate:	3007
Positive control well median absolute deviation, by plate:	164.569
Negative control well median, by plate:	110.860784177171
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.11420865277645
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.6852519166587

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Raynor K, Reisine T. Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. J Pharmacol Exp Ther. 1989 Nov;251(2):510-7. PubMed PMID: 2572690.|Reubi JC, Perrin MH, Rivier JE, Vale W. High affinity binding sites for a somatostatin-28 analog in rat brain. Life Sci. 1981 May 11;28(19):2191-8. PubMed PMID: 6114372.|Czernik AJ, Petrack B. Somatostatin receptor binding in rat cerebral cortex. Characterization using a nonreducible somatostatin analog. J Biol Chem. 1983 May 10;258(9):5525-30. PubMed PMID: 6133870.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Raynor K Reisine T Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain J Pharmacol Exp Ther 1989 Nov 251 2 510 7 PubMed PMID 2572690 Reubi JC Perrin MH Rivier JE Vale W High affinity binding sites for a somatostatin 28 analog in rat brain Life Sci 1981 May 11 28 19 2191 8 PubMed PMID 6114372 Czernik AJ Petrack B Somatostatin receptor binding in rat cerebral cortex Characterization using a nonreducible somatostatin analog J Biol Chem 1983 May 10 258 9 5525 30 PubMed PMID 6133870 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rTRH

Assay Title: Novascreen rat unspecified thyrotropin releasing hormone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rTRH was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rTRH, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Trhr. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rTRH, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rTRH assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rTRH is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 4 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1586.97
Neutral control median absolute deviation, by plate:	47.6285
Positive control well median response value, by plate:	535.34
Positive control well median absolute deviation, by plate:	25.8862
Negative control well median, by plate:	144.083233488093
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.77
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.36
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.34
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.91726902114934
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.503614126896

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Burt DR, Snyder SH. Thyrotropin releasing hormone (TRH): apparent receptor binding in rat brain membranes. Brain Res. 1975 Aug 8;93(2):309-28. PubMed PMID: 169955.|Taylor RL, Burt DR. Preparation of 3H-[3-M3-His2]TRH as an improved ligand for TRH receptors. Neuroendocrinology. 1981 May;32(5):310-6. PubMed PMID: 6264336.|Simasko SM, Horita A. Characterization and distribution of 3H-(3MeHis2)thyrotropin releasing hormone receptors in rat brain. Life Sci. 1982 May 24;30(21):1793-9. PubMed PMID: 6285110.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Burt DR Snyder SH Thyrotropin releasing hormone TRH apparent receptor binding in rat brain membranes Brain Res 1975 Aug 8 93 2 309 28 PubMed PMID 169955 Taylor RL

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rV1

Assay Title: Novascreen rat unspecified arginine vasopressin receptor 1A assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rV1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rV1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Avpr1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "rhodopsin-like receptor".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rV1, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rV1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rV1 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat liver membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3379.87
Neutral control median absolute deviation, by plate:	58.5805
Positive control well median response value, by plate:	644.83
Positive control well median absolute deviation, by plate:	17.9988
Negative control well median, by plate:	93.7940078546977
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-39.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.09006854850897
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gopalakrishnan V, Triggle CR, Sulakhe PV, McNeill JR. Characterization of a specific, high affinity [3H]arginine8 vasopressin-binding site on liver microsomes from different strains of rat and the role of magnesium. Endocrinology. 1986 Mar;118(3):990-7. PubMed PMID: 3004905.|Dorsa DM, Petracca FM, Baskin DG, Cornett LE. Localization and characterization of vasopressin-binding sites in the amygdala of the rat brain. J Neurosci. 1984 Jul;4(7):1764-70. PubMed PMID: 6330316.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_GPCR_rVIP_NonSelective

Assay Title: Novascreen rat unspecified vasoactive intestinal peptide receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_GPCR_rVIP_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_GPCR_rVIP_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Vipr1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "gpcr" intended target family, where the subfamily is "secretin receptor".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_GPCR_rVIP_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_GPCR_rVIP_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_GPCR_rVIP_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7188
Neutral control median absolute deviation, by plate:	57.8214
Positive control well median response value, by plate:	3316
Positive control well median absolute deviation, by plate:	59.304
Negative control well median, by plate:	91.3447685203992
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-65.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.49
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.72168446601941
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	34.3301067961165

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Korman LY, Sayadi H, Bass B, Moody TW, Harmon JW. Distribution of vasoactive intestinal polypeptide and substance P receptors in human colon and small intestine. Dig Dis Sci. 1989 Jul;34(7):1100-8. PubMed PMID: 2472937.|Ogawa N, Mizuno S, Mori A, Nukina I, Yanaihara N. Properties and distribution of vasoactive intestinal polypeptide receptors in the rat brain. Peptides. 1985;6 Suppl 1:103-9. PubMed PMID: 2995938.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Korman LY Sayadi H Bass B Moody TW Harmon JW Distribution of vasoactive intestinal polypeptide and substance P receptors in human colon and small intestine Dig Dis Sci 1989 Jul 34 7 1100 8 PubMed PMID 2472937 Ogawa N Mizuno S Mori A Nukina I Yanaihara N Properties and distribution of vasoactive intestinal polypeptide receptors in the rat brain Peptides 1985 6 Suppl 1 103 9 PubMed PMID 2995938 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_hKhERGCh

Assay Title: Novascreen human unspecified potassium voltage-gated channel, subfamily H (eag-related), member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_hKhERGCh was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_hKhERGCh, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene KCNH2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "potassium channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_IC_hKhERGCh, is one of 1 assay component(s) measured or calculated from the NVS_IC_hKhERGCh assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_hKhERGCh is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	16573.4
Neutral control median absolute deviation, by plate:	603.863
Positive control well median response value, by plate:	3450.33
Positive control well median absolute deviation, by plate:	107.34
Negative control well median, by plate:	94.6743566881382
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.19
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.08213152643258
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.4927891585955

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Zhou Z, Vorperian VR, Gong Q, Zhang S, January CT. Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole. J Cardiovasc Electrophysiol. 1999 Jun;10(6):836-43. PubMed PMID: 10376921.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Zhou Z Vorperian VR Gong Q Zhang S January CT Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole J Cardiovasc Electrophysiol 1999 Jun 10 6 836 43 PubMed PMID 10376921 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Zhou Z Vorperian VR Gong Q Zhang S January CT Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole J Cardiovasc Electrophysiol 1999 Jun 10 6 836 43 PubMed PMID 10376921 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rCaBTZCHL

Assay Title: Novascreen rat unspecified calcium channel, voltage-dependent, P/Q type, alpha 1A subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rCaBTZCHL was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rCaBTZCHL, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cacna1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "calcium channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rCaBTZCHL, is one of 1 assay component(s) measured or calculated from the NVS_IC_rCaBTZCHL assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rCaBTZCHL is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3577.3
Neutral control median absolute deviation, by plate:	134.353
Positive control well median response value, by plate:	1164.57
Positive control well median absolute deviation, by plate:	59.3633
Negative control well median, by plate:	114.145104061299
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.73
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-18.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.93611263599688
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	35.6166758159813

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Schoemaker H, Langer SZ. [3H]diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex. Eur J Pharmacol. 1985 May 8;111(2):273-7. PubMed PMID: 2410283.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Schoemaker H Langer SZ 3H diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex Eur J Pharmacol 1985 May 8 111 2 273 7 PubMed PMID 2410283 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Schoemaker H Langer SZ 3H diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex Eur J Pharmacol 1985 May 8 111 2 273 7 PubMed PMID 2410283 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011

Jan 18 PubMed PMID 21251949 Schoemaker H Langer SZ 3H diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex Eur J Pharmacol 1985 May 8 111 2 273 7 PubMed PMID 2410283 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rCaChN

Assay Title: Novascreen rat unspecified calcium channel, voltage-dependent, N type, alpha 1B subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rCaChN was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rCaChN, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cacna1b. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "calcium channel".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rCaChN, is one of 1 assay component(s) measured or calculated from the NVS_IC_rCaChN assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rCaChN is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5573.5
Neutral control median absolute deviation, by plate:	183.101
Positive control well median response value, by plate:	946.5
Positive control well median absolute deviation, by plate:	35.5824
Negative control well median, by plate:	93.2838080155913
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-25
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.98268929651998
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.8961357791199

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rCaDHPRCh_L

Assay Title: Novascreen rat unspecified calcium channel, voltage-dependent, P/Q type, alpha 1A subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rCaDHPRCh_L was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rCaDHPRCh_L, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Cacna1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "calcium channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rCaDHPRCh_L, is one of 1 assay component(s) measured or calculated from the NVS_IC_rCaDHPRCh_L assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rCaDHPRCh_L is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3144.91
Neutral control median absolute deviation, by plate:	72.9662
Positive control well median response value, by plate:	745.79
Positive control well median absolute deviation, by plate:	31.268
Negative control well median, by plate:	104.889188180073
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-31.39
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.23
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.63292901835292
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.7975741101175

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Gould RJ, Murphy KM, Snyder SH. Tissue heterogeneity of calcium channel antagonist binding sites labeled by [3H]nitrendipine. Mol Pharmacol. 1984 Mar;25(2):235-41. PubMed PMID: 6422256.|Ehlert FJ, Roeske WR, Itoga E, Yamamura HI. The binding of [3H]nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex, and ileum of rats. Life Sci. 1982 Jun 21;30(25):2191-202. PubMed PMID: 7109844.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rKAR

Assay Title: Novascreen rat unspecified glutamate receptor, ionotropic, kainate 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rKAR was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rKAR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Grik1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rKAR, is one of 1 assay component(s) measured or calculated from the NVS_IC_rKAR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rKAR is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9898.82
Neutral control median absolute deviation, by plate:	106.169
Positive control well median response value, by plate:	643.78
Positive control well median absolute deviation, by plate:	15.2263
Negative control well median, by plate:	96.6283786141496
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-66
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-74.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.88358615218036
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

London ED, Coyle JT. Specific binding of [3H]kainic acid to receptor sites in rat brain. Mol Pharmacol. 1979 May;15(3):492-505. PubMed PMID: 492142.|Hall RA, Kessler M, Lynch G. Kainate binding to the AMPA receptor in rat brain. Neurochem Res. 1994 Jun;19(6):777-82. PubMed PMID: 7520541.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|London ED Coyle JT Specific binding of 3H kainic acid to receptor sites in rat brain Mol Pharmacol 1979 May 15 3 492 505 PubMed PMID 492142 Hall RA Kessler M Lynch G Kainate binding to the AMPA receptor in rat brain Neurochem Res 1994 Jun 19 6 777 82 PubMed PMID 7520541 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rKATPCh

Assay Title: Novascreen rat unspecified potassium inwardly-rectifying channel, subfamily J, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rKATPCh was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rKATPCh, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Kcnj1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "potassium channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rKATPCh, is one of 1 assay component(s) measured or calculated from the NVS_IC_rKATPCh assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rKATPCh is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2653.86
Neutral control median absolute deviation, by plate:	77.2657
Positive control well median response value, by plate:	280.45
Positive control well median absolute deviation, by plate:	16.8868
Negative control well median, by plate:	99.3924288220846
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.85
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-32.33
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.86371786871514
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.1823072122908

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4. Assay Documentation

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Bernardi H, Fosset M, Lazdunski M. Characterization, purification, and affinity labeling of the brain [3H]glibenclamide-binding protein, a putative neuronal ATP-regulated K+ channel. Proc Natl Acad Sci U S A. 1988 Dec;85(24):9816-20. PubMed PMID: 3144003; PubMed Central PMCID: PMC282872.|Geisen K, Hitzel V, Okomonopoulos R, PÃ¼nter J, Weyer R, Summ HD. Inhibition of 3H-glibenclamide binding to sulfonylurea receptors by oral antidiabetics. Arzneimittelforschung. 1985;35(4):707-12. PubMed PMID: 3925960.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rKCaCh

Assay Title: Novascreen rat unspecified potassium intermediate/small conductance calcium-activated channel, subfamily N, member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rKCaCh was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rKCaCh, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Kcnn1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "potassium channel".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rKCaCh, is one of 1 assay component(s) measured or calculated from the NVS_IC_rKCaCh assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rKCaCh is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6205
Neutral control median absolute deviation, by plate:	136.399
Positive control well median response value, by plate:	1735
Positive control well median absolute deviation, by plate:	68.1996
Negative control well median, by plate:	95.1792971438809
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.28
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.86482216142271
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	35.1889329685363

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Habermann E, Fischer K. Bee venom neurotoxin (apamin): iodine labeling and characterization of binding sites. Eur J Biochem. 1979 Mar;94(2):355-64. PubMed PMID: 428392.|Seagar MJ, Marqueze B, Couraud F. Solubilization of the apamin receptor associated with a calcium-activated potassium channel from rat brain. J Neurosci. 1987 Feb;7(2):565-70. PubMed PMID: 2434630.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_IC_rNaCh_site2

Assay Title: Novascreen rat unspecified sodium channel, voltage-gated, type I, alpha subunit assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_IC_rNaCh_site2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_IC_rNaCh_site2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Scn1a. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "sodium channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_IC_rNaCh_site2, is one of 1 assay component(s) measured or calculated from the NVS_IC_rNaCh_site2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_IC_rNaCh_site2 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2863.08
Neutral control median absolute deviation, by plate:	80.305
Positive control well median response value, by plate:	485.57
Positive control well median absolute deviation, by plate:	20.5785
Negative control well median, by plate:	110.24352864701
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-22
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-29.85
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.26674364194948
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.6004618516969

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Creveling CR, McNeal ET, Daly JW, Brown GB. Batrachotoxin-induced depolarization and [3H]batrachotoxinin-a 20 alpha-benzoate binding in a vesicular preparation from guinea pig cerebral cortex. Mol Pharmacol. 1983 Mar;23(2):350-8. PubMed PMID: 6300644.|Trainer VL, Moreau E, Guedin D, Baden DG, Catterall WA. Neurotoxin binding and allosteric modulation at receptor sites 2 and 5 on purified and reconstituted rat brain sodium channels. J Biol Chem. 1993 Aug 15;268(23):17114-9. PubMed PMID: 8394327.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_bGABAR_Agonist

Assay Title: Novascreen bovine unspecified gamma-aminobutyric acid (GABA) A receptor, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_bGABAR_Agonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_bGABAR_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene GABRA1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_bGABAR_Agonist, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_bGABAR_Agonist assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_bGABAR_Agonist is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine cerebellar membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1948.5
Neutral control median absolute deviation, by plate:	51.8465
Positive control well median response value, by plate:	191.16
Positive control well median absolute deviation, by plate:	18.1989
Negative control well median, by plate:	94.4758826621336
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-29
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-38.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.63056142764063
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.7833685658438

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Enna SJ, Collins JF, Snyder SH. Stereospecificity and structure--activity requirements of GABA receptor binding in rat brain. Brain Res. 1977 Mar 18;124(1):185-90. PubMed PMID: 191147.|Falch E, Hedegaard A, Nielsen L, Jensen BR, Hjeds H, Krogsgaard-Larsen P. Comparative stereostructure-activity studies on GABAA and GABAB receptor sites and GABA uptake using rat brain membrane preparations. J Neurochem. 1986 Sep;47(3):898-903. PubMed PMID: 3016189.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_bGABARa1

Assay Title: Novascreen bovine unspecified gamma-aminobutyric acid (GABA) A receptor, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_bGABARa1 was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_bGABARa1, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene GABRA1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_bGABARa1, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_bGABARa1 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_bGABARa1 is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine cortical membranes in a tissue-based cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4597.8
Neutral control median absolute deviation, by plate:	94.1006
Positive control well median response value, by plate:	632.83
Positive control well median absolute deviation, by plate:	27.371
Negative control well median, by plate:	100.788056506963
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-37.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.76732192385783
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Sweetnam PM, Tallman JF. Regional difference in brain benzodiazepine receptor carbohydrates. Mol Pharmacol. 1986 Mar;29(3):299-306. PubMed PMID: 3005837.|Zarkovsky AM. Bicuculline-sensitive and insensitive effects of THIP on the binding of [3H]flunitrazepam. Neuropharmacology. 1987 Jul;26(7A):737-41. PubMed PMID: 3627382.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Sweetnam PM Tallman JF Regional difference in brain benzodiazepine receptor carbohydrates Mol Pharmacol 1986 Mar 29 3 299 306 PubMed PMID 3005837 Zarkovsky AM Bicuculline sensitive and insensitive effects of THIP on the binding of 3H flunitrazepam Neuropharmacology 1987 Jul 26 7A 737 41 PubMed PMID 3627382 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_bGABARa5

Assay Title: Novascreen bovine unspecified gamma-aminobutyric acid (GABA) A receptor, alpha 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_bGABARa5 was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_bGABARa5, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene GABRA5. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_bGABARa5, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_bGABARa5 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_bGABARa5 is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine hippocampal membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1228.97
Neutral control median absolute deviation, by plate:	47.0132
Positive control well median response value, by plate:	212.69
Positive control well median absolute deviation, by plate:	17.9246
Negative control well median, by plate:	99.0921863179509
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.82
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.88574222317201
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.3144533390321

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Li M, Szabo A, Rosenberg HC. Evaluation of native GABA(A) receptors containing an alpha 5 subunit. Eur J Pharmacol. 2001 Feb 9;413(1):63-72. PubMed PMID: 11173064.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Li M Szabo A Rosenberg HC Evaluation of native GABA A receptors containing an alpha 5 subunit Eur J Pharmacol 2001 Feb 9 413 1 63 72 PubMed PMID 11173064 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Li M Szabo A Rosenberg HC Evaluation of native GABA A receptors containing an alpha 5 subunit Eur J Pharmacol 2001 Feb 9 413 1 63 72 PubMed PMID 11173064 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_h5HT3

Assay Title: Novascreen human unspecified 5-hydroxytryptamine (serotonin) receptor 3A, ionotropic assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_h5HT3 was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_h5HT3, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene HTR3A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_LGIC_h5HT3, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_h5HT3 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_h5HT3 is a biochemical, single-readout assay that uses extracted gene-proteins from HEK293 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3042.64
Neutral control median absolute deviation, by plate:	206.111
Positive control well median response value, by plate:	268.67
Positive control well median absolute deviation, by plate:	20.8157
Negative control well median, by plate:	102.421595871378
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-12.09
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.31405014212946
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.8843008527768

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Tyers MB. 5-HT3 receptors and the therapeutic potential of 5-HT3 receptor antagonists. Therapie. 1991 Nov-Dec;46(6):431-5. Review. PubMed PMID: 1840227.|Lummis SC, Kilpatrick GJ, Martin IL. Characterization of 5-HT3 receptors in intact N1E-115 neuroblastoma cells. Eur J Pharmacol. 1990 Sep 18;189(2-3):223-7. PubMed PMID: 2253704.|Hoyer D, Neijt HC. Identification of serotonin 5-HT3 recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. Mol Pharmacol. 1988 Mar;33(3):303-9. PubMed PMID: 3352595.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Tyers MB 5 HT3 receptors and the therapeutic potential of 5 HT3 receptor antagonists Therapie 1991 Nov Dec 46 6 431 5 Review PubMed PMID 1840227 Lummis SC Kilpatrick GJ Martin IL Characterization of 5 HT3 receptors in intact N1E 115 neuroblastoma cells Eur J Pharmacol 1990 Sep 18 189 2 3 223 7 PubMed PMID 2253704 Hoyer D Neijt HC Identification of serotonin 5 HT3 recognition sites in membranes of N1E 115 neuroblastoma cells by radioligand binding Mol Pharmacol 1988 Mar 33 3 303 9 PubMed PMID 3352595 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_hNNR_NBungSens

Assay Title: Novascreen human unspecified cholinergic receptor, nicotinic, alpha 2 (neuronal) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_hNNR_NBungSens was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_hNNR_NBungSens, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene CHRNA2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_hNNR_NBungSens, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_hNNR_NBungSens assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_hNNR_NBungSens is a biochemical, single-readout assay that uses extracted gene-proteins from Human neuroblastoma membrane cells in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2218.04
Neutral control median absolute deviation, by plate:	50.1712
Positive control well median response value, by plate:	155.505
Positive control well median absolute deviation, by plate:	14.0328
Negative control well median, by plate:	103.096780595682
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.9
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.25779400021618
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Luetje CW, Patrick J, SÃ©guÃ©la P. Nicotine receptors in the mammalian brain. FASEB J. 1990 Jul;4(10):2753-60. Review. PubMed PMID: 2197155.|Perry DC, Kellar KJ. [3H]epibatidine labels nicotinic receptors in rat brain: an autoradiographic study. J Pharmacol Exp Ther. 1995 Nov;275(2):1030-4. PubMed PMID: 7473129.|Fisher M, Huangfu D, Shen TY, Guyenet PG. Epibatidine, an alkaloid from the poison frog Epipedobates tricolor, is a powerful ganglionic depolarizing agent. J Pharmacol Exp Ther. 1994 Aug;270(2):702-7. PubMed PMID: 8071862.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Luetje CW Patrick J S gu la P Nicotine receptors in the mammalian brain FASEB J 1990 Jul 4 10 2753 60 Review PubMed PMID 2197155 Perry DC Kellar KJ 3H epibatidine labels nicotinic receptors in rat brain an autoradiographic study J Pharmacol Exp Ther 1995 Nov 275 2 1030 4 PubMed PMID 7473129 Fisher M Huangfu D Shen TY Guyenet PG Epibatidine an alkaloid from the poison frog Epipedobates tricolor is a powerful ganglionic depolarizing agent J Pharmacol Exp Ther 1994 Aug 270 2 702 7 PubMed PMID 8071862 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rAMPA

Assay Title: Novascreen rat unspecified glutamate receptor, ionotropic, AMPA 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rAMPA was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rAMPA, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Gria1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rAMPA, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rAMPA assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rAMPA is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5790.2
Neutral control median absolute deviation, by plate:	126.555
Positive control well median response value, by plate:	666.905
Positive control well median absolute deviation, by plate:	29.6372
Negative control well median, by plate:	93.7543043070085
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-36
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-37.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.12
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.00089273658152
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Murphy DE, Snowhill EW, Williams M. Characterization of quisqualate recognition sites in rat brain tissue using DL-[3H]alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and a filtration assay. Neurochem Res. 1987 Sep;12(9):775-81. PubMed PMID: 2890112.|Morgan RC, Mercer LD, Cincotta M, Beart PM. Binding of [(3)H]AMPA to non-chaotrope, non-detergent treated rat synaptic membranes: Characteristics and lack of effect of barbiturates. Neurochem Int. 1991;18(1):75-84. PubMed PMID: 20504679.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Murphy DE Snowhill EW Williams M Characterization of quisqualate recognition sites in rat brain tissue using DL 3H alpha amino 3 hydroxy 5 methylisoxazole 4 propionic acid AMPA and a filtration assay Neurochem Res 1987 Sep 12 9 775 81 PubMed PMID 2890112 Morgan RC Mercer LD Cincotta M Beart PM Binding of 3 H AMPA to non chaotrope non detergent treated rat synaptic membranes Characteristics and lack of effect of barbiturates Neurochem Int 1991 18 1 75 84 PubMed PMID 20504679 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rGABAR_NonSelective

Assay Title: Novascreen rat unspecified gamma-aminobutyric acid (GABA) A receptor, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rGABAR_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rGABAR_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Gabra1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rGABAR_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rGABAR_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rGABAR_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Rat whole brain in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4121.08
Neutral control median absolute deviation, by plate:	111.284
Positive control well median response value, by plate:	718.37
Positive control well median absolute deviation, by plate:	47.1319
Negative control well median, by plate:	110.443846534419
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.79
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-19
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.77
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.26394263262771
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.5836557957663

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Enna SJ, Collins JF, Snyder SH. Stereospecificity and structure--activity requirements of GABA receptor binding in rat brain. Brain Res. 1977 Mar 18;124(1):185-90. PubMed PMID: 191147.|Falch E, Hedegaard A, Nielsen L, Jensen BR, Hjeds H, Krogsgaard-Larsen P. Comparative stereostructure-activity studies on GABAA and GABAB receptor sites and GABA uptake using rat brain membrane preparations. J Neurochem. 1986 Sep;47(3):898-903. PubMed PMID: 3016189.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rGABARa6

Assay Title: Novascreen rat unspecified gamma-aminobutyric acid (GABA) A receptor, alpha 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rGABARa6 was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rGABARa6, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Gabra6. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rGABARa6, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rGABARa6 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rGABARa6 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cerebellar membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2666.15
Neutral control median absolute deviation, by plate:	55.5975
Positive control well median response value, by plate:	215.035
Positive control well median absolute deviation, by plate:	7.76141
Negative control well median, by plate:	96.0816673031032
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.91
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-42
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-49.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.41731493040414
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.5038895824248

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

MÃ¤kelÃ¤ R, Uusi-Oukari M, Homanics GE, Quinlan JJ, Firestone LL, Wisden W, Korpi ER. Cerebellar gamma-aminobutyric acid type A receptors: pharmacological subtypes revealed by mutant mouse lines. Mol Pharmacol. 1997 Sep;52(3):380-8. PubMed PMID: 9281599.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|M kel R Uusi Oukari M Homanics GE Quinlan JJ Firestone LL Wisden W Korpi ER Cerebellar gamma aminobutyric acid type A receptors pharmacological subtypes revealed by mutant mouse lines Mol Pharmacol 1997 Sep 52 3 380 8 PubMed PMID 9281599 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rGluNMDA_Agonist

Assay Title: Novascreen rat unspecified glutamate receptor, ionotropic, N-methyl D-aspartate 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rGluNMDA_Agonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rGluNMDA_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Grin1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rGluNMDA_Agonist, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rGluNMDA_Agonist assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rGluNMDA_Agonist is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3482.19
Neutral control median absolute deviation, by plate:	100.357
Positive control well median response value, by plate:	873.165
Positive control well median absolute deviation, by plate:	43.2623
Negative control well median, by plate:	89.2887705127464
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-29
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-36.29
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.25
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.90079310386768
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.4047586232061

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Lehmann J, Hutchison AJ, McPherson SE, Mondadori C, Schmutz M, Sinton CM, Tsai C, Murphy DE, Steel DJ, Williams M, et al. CGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. J Pharmacol Exp Ther. 1988 Jul;246(1):65-75. PubMed PMID: 2899170.|Murphy DE, Schneider J, Boehm C, Lehmann J, Williams M. Binding of [3H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat brain membranes: a selective, high-affinity ligand for N-methyl-D-aspartate receptors. J Pharmacol Exp Ther. 1987 Mar;240(3):778-84. PubMed PMID: 3031274.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Lehmann J Hutchison AJ McPherson SE Mondadori C Schmutz M Sinton CM Tsai C Murphy DE Steel DJ Williams M et al CGS 19755 a selective and competitive N methyl D aspartate type excitatory amino acid receptor antagonist J Pharmacol Exp Ther 1988 Jul 246 1 65 75 PubMed PMID 2899170 Murphy DE Schneider J Boehm C Lehmann J Williams M Binding of 3H 3 2 carboxypiperazin 4 yl propyl 1 phosphonic acid to rat brain membranes a selective high affinity ligand for N methyl D aspartate receptors J Pharmacol Exp Ther 1987 Mar 240 3 778 84 PubMed PMID 3031274 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rGluNMDA_MK801_Agonist

Assay Title: Novascreen rat unspecified glutamate receptor, ionotropic, N-methyl D-aspartate 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rGluNMDA_MK801_Agonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rGluNMDA_MK801_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Grin1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rGluNMDA_MK801_Agonist, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rGluNMDA_MK801_Agonist assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rGluNMDA_MK801_Agonist is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7595.21
Neutral control median absolute deviation, by plate:	65.9609
Positive control well median response value, by plate:	668.36
Positive control well median absolute deviation, by plate:	58.2217
Negative control well median, by plate:	98.9922812178967
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.94
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-63
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-112.78
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.37572328026236
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Javitt DC, Zukin SR. Biexponential kinetics of [3H]MK-801 binding: evidence for access to closed and open N-methyl-D-aspartate receptor channels. Mol Pharmacol. 1989 Apr;35(4):387-93. PubMed PMID: 2468076.|Foster AC, Wong EH. The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. Br J Pharmacol. 1987 Jun;91(2):403-9. PubMed PMID: 2886170; PubMed Central PMCID: PMC1853511.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Javitt DC Zukin SR Biexponential kinetics of 3H MK 801 binding evidence for access to closed and open N methyl D aspartate receptor channels Mol Pharmacol 1989 Apr 35 4 387 93 PubMed PMID 2468076 Foster AC Wong EH The novel anticonvulsant MK 801 binds to the activated state of the N methyl D aspartate receptor in rat brain Br J Pharmacol 1987 Jun 91 2 403 9 PubMed PMID 2886170 PubMed Central PMCID PMC1853511 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rGlyRStrySens

Assay Title: Novascreen rat unspecified glycine receptor, alpha 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rGlyRStrySens was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rGlyRStrySens, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Glra1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rGlyRStrySens, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rGlyRStrySens assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rGlyRStrySens is a biochemical, single-readout assay that uses extracted gene-proteins from Rat spinal cord membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	9570.56
Neutral control median absolute deviation, by plate:	310.961
Positive control well median response value, by plate:	1587.9
Positive control well median absolute deviation, by plate:	57.2432
Negative control well median, by plate:	92.7894947122006
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.76346792996833
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.58080757981

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Ruiz-GÃ³mez A, GarcÃ-a-Calvo M, VÃ¡zquez J, MarvizÃ³n JC, Valdivieso F, Mayor F Jr. Thermodynamics of agonist and antagonist interaction with the strychnine-sensitive glycine receptor. J Neurochem. 1989 Jun;52(6):1775-80. PubMed PMID: 2542450.|Young AB and Snyder SH. Strychnine Binding in Rat Spinal Cord Membranes Associated with the Synaptic Glycine Receptor: Cooperativity of Glycine Interactions. Mol. Pharmacol. 1974. 10: 790-809|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Ruiz G mez A Garc a Calvo M V zquez J Marviz n JC Valdivieso F Mayor F Jr Thermodynamics of agonist and antagonist interaction with the strychnine sensitive glycine receptor J Neurochem 1989 Jun 52 6 1775 80 PubMed PMID 2542450 Young AB and Snyder SH Strychnine Binding in Rat Spinal Cord Membranes Associated with the Synaptic Glycine Receptor Cooperativity of Glycine Interactions Mol Pharmacol 1974 10 790 809 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_LGIC_rNNR_BungSens

Assay Title: Novascreen rat unspecified cholinergic receptor, nicotinic, alpha 7 (neuronal) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_LGIC_rNNR_BungSens was analyzed into 1 assay endpoint. This assay endpoint, NVS_LGIC_rNNR_BungSens, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Chrna7. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "ion channel" intended target family, where the subfamily is "ligand-gated ion channel".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_LGIC_rNNR_BungSens, is one of 1 assay component(s) measured or calculated from the NVS_LGIC_rNNR_BungSens assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_LGIC_rNNR_BungSens is a biochemical, single-readout assay that uses extracted gene-proteins from Rat cortical membranes in a tissue-based cell-free assay.Measurements were taken 2.5 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	15955.8
Neutral control median absolute deviation, by plate:	274.503
Positive control well median response value, by plate:	3780.05
Positive control well median absolute deviation, by plate:	87.9923
Negative control well median, by plate:	103.103767186633
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.86
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-43.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.27
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.09361687982466
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Luetje CW, Patrick J, SÃ©guÃ©la P. Nicotine receptors in the mammalian brain. FASEB J. 1990 Jul;4(10):2753-60. Review. PubMed PMID: 2197155.|Perry DC, Kellar KJ. [3H]epibatidine labels nicotinic receptors in rat brain: an autoradiographic study. J Pharmacol Exp Ther. 1995 Nov;275(2):1030-4. PubMed PMID: 7473129.|Fisher M, Huangfu D, Shen TY, Guyenet PG. Epibatidine, an alkaloid from the poison frog Epipedobates tricolor, is a powerful ganglionic depolarizing agent. J Pharmacol Exp Ther. 1994 Aug;270(2):702-7. PubMed PMID: 8071862.|Meyer EM, Kuryatov A, Gerzanich V, Lindstrom J, Papke RL. Analysis of 3-(4-hydroxy, 2-Methoxybenzylidene)anabaseine selectivity and activity at human and rat alpha-7 nicotinic receptors. J Pharmacol Exp Ther. 1998 Dec;287(3):918-25. PubMed PMID: 9864273.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Luetje CW Patrick J S gu la P Nicotine receptors in the mammalian brain FASEB J 1990 Jul 4 10 2753 60 Review PubMed PMID 2197155 Perry DC Kellar KJ 3H epibatidine labels nicotinic receptors in rat brain an autoradiographic study J Pharmacol Exp Ther 1995 Nov 275 2 1030 4 PubMed PMID 7473129 Fisher M Huangfu D Shen TY Guyenet PG Epibatidine an alkaloid from the poison frog Epipedobates tricolor is a powerful ganglionic depolarizing agent J Pharmacol Exp Ther 1994 Aug 270 2 702 7 PubMed PMID 8071862 Meyer EM Kuryatov A Gerzanich V Lindstrom J Papke RL Analysis of 3 4 hydroxy 2 Methoxybenzylidene anabaseine selectivity and activity at human and rat alpha 7 nicotinic receptors J Pharmacol Exp Ther 1998 Dec 287 3 918 25 PubMed PMID 9864273 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV

Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

<u>Date of Assay Document Creation:</u>

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_MP_hPBR

Assay Title: Novascreen human unspecified translocator protein (18kDa) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_MP_hPBR was analyzed into 1 assay endpoint. This assay endpoint, NVS_MP_hPBR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene TSPO. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "cholesterol transporter".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_MP_hPBR, is one of 1 assay component(s) measured or calculated from the NVS_MP_hPBR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_MP_hPBR is a biochemical, single-readout assay that uses extracted gene-proteins from Human colonic cell membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	7844.28
Neutral control median absolute deviation, by plate:	223.873
Positive control well median response value, by plate:	1564.84
Positive control well median absolute deviation, by plate:	62.3433
Negative control well median, by plate:	116.551785817625
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.89
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-34
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-39.14
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.67709020137799
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.0625412082679

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Skowro?ski R, Fanestil DD, Beaumont K. Photoaffinity labeling of peripheral-type benzodiazepine receptors in rat kidney mitochondria with [3H]PK 14105. Eur J Pharmacol. 1988 Mar 29;148(2):187-93. PubMed PMID: 2837399.|Rao VL, Audet R, Therrien G, Butterworth RF. Tissue-specific alterations of binding sites for peripheral-type benzodiazepine receptor ligand [3H]PK11195 in rats following portacaval anastomosis. Dig Dis Sci. 1994 May;39(5):1055-63. PubMed PMID: 8174418.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Skowro ski R Fanestil DD Beaumont K Photoaffinity labeling of peripheral type benzodiazepine receptors in rat kidney mitochondria with 3H PK 14105 Eur J Pharmacol 1988 Mar 29 148 2 187 93 PubMed PMID 2837399 Rao VL Audet R Therrien G Butterworth RF Tissue specific alterations of binding sites for peripheral type benzodiazepine receptor ligand 3H PK11195 in rats following portacaval anastomosis Dig Dis Sci 1994 May 39 5 1055 63 PubMed PMID 8174418 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_MP_rPBR

Assay Title: Novascreen rat unspecified translocator protein assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_MP_rPBR was analyzed into 1 assay endpoint. This assay endpoint, NVS_MP_rPBR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Tspo. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "cholesterol transporter".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_MP_rPBR, is one of 1 assay component(s) measured or calculated from the NVS_MP_rPBR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_MP_rPBR is a biochemical, single-readout assay that uses extracted gene-proteins from Rat kidney membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6347.33
Neutral control median absolute deviation, by plate:	238.765
Positive control well median response value, by plate:	1583.42
Positive control well median absolute deviation, by plate:	65.5161
Negative control well median, by plate:	111.183097035896
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.79
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.59
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.26
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.17700292198745
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.0620175319247

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Skowro?ski R, Fanestil DD, Beaumont K. Photoaffinity labeling of peripheral-type benzodiazepine receptors in rat kidney mitochondria with [3H]PK 14105. Eur J Pharmacol. 1988 Mar 29;148(2):187-93. PubMed PMID: 2837399.|Rao VL, Audet R, Therrien G, Butterworth RF. Tissue-specific alterations of binding sites for peripheral-type benzodiazepine receptor ligand [3H]PK11195 in rats following portacaval anastomosis. Dig Dis Sci. 1994 May;39(5):1055-63. PubMed PMID: 8174418.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Skowro ski R Fanestil DD Beaumont K Photoaffinity labeling of peripheral type benzodiazepine receptors in rat kidney mitochondria with 3H PK 14105 Eur J Pharmacol 1988 Mar 29 148 2 187 93 PubMed PMID 2837399 Rao VL Audet R Therrien G Butterworth RF Tissue specific alterations of binding sites for peripheral type benzodiazepine receptor ligand 3H PK11195 in rats following portacaval anastomosis Dig Dis Sci 1994 May 39 5 1055 63 PubMed PMID 8174418 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_bPR

Assay Title: Novascreen bovine unspecified progesterone receptor-like assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_bPR was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_bPR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene PGR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_NR_bPR, is one of 1 assay component(s) measured or calculated from the NVS_NR_bPR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_bPR is a biochemical, single-readout assay that uses extracted gene-proteins from Bovine uterine membranes in a tissue-based cell-free assay.Measurements were taken 18 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3567.65
Neutral control median absolute deviation, by plate:	96.3838
Positive control well median response value, by plate:	293.17
Positive control well median absolute deviation, by plate:	15.1818
Negative control well median, by plate:	120.869876836606
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-29
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-33.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.15561891578676
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.9337134947206

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Traish AM, MÃ¼ller RE, Wotiz HH. Binding of 7 alpha, 17 alpha-dimethyl-19-nortestosterone (mibolerone) to androgen and progesterone receptors in human and animal tissues. Endocrinology. 1986 Apr;118(4):1327-33. PubMed PMID: 2419120.|Haji M, Kato KI, Nawata H, Ibayashi H. Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat. Brain Res. 1981 Jan 12;204(2):373-86. PubMed PMID: 6780133.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Traish AM M ller RE Wotiz HH Binding of 7 alpha 17 alpha dimethyl 19 nortestosterone mibolerone to androgen and progesterone receptors in human and animal tissues Endocrinology 1986 Apr 118 4 1327 33 PubMed PMID 2419120 Haji M Kato KI Nawata H Ibayashi H Age related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat Brain Res 1981 Jan 12 204 2 373 86 PubMed PMID 6780133 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Traish AM M ller RE Wotiz HH Binding of 7 alpha 17 alpha dimethyl 19 nortestosterone mibolerone to androgen and progesterone receptors in human and animal tissues Endocrinology 1986 Apr 118 4 1327 33 PubMed PMID 2419120 Haji M Kato KI Nawata H Ibayashi H Age related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat Brain Res 1981 Jan 12 204 2 373 86 PubMed PMID 6780133 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hCAR_Agonist

Assay Title: Novascreen human unspecified nuclear receptor subfamily 1, group I, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hCAR_Agonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hCAR_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NR1I3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hCAR_Agonist, is one of 1 assay component(s) measured or calculated from the NVS_NR_hCAR_Agonist assay. It is designed to make measurements of fluorescent resonance energy transfer, a form of binding reporter, as detected with fluorescent resonance energy transfer signals by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hCAR_Agonist is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.581
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	2.279
Positive control well median absolute deviation, by plate:	0.0830256
Negative control well median, by plate:	4883.18584070797
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	24
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	257.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	4.47
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	140.993342409803
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	845.960054458818

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Maglich JM, Parks DJ, Moore LB, Collins JL, Goodwin B, Billin AN, Stoltz CA, Kliewer SA, Lambert MH, Willson TM, Moore JT. Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. J Biol Chem. 2003 May 9;278(19):17277-83. Epub 2003 Feb 27. PubMed PMID: 12611900.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Maglich JM Parks DJ Moore LB Collins JL Goodwin B Billin AN Stoltz CA Kliewer SA Lambert MH Willson TM Moore JT Identification of a novel human constitutive androstane receptor CAR agonist and its use in the identification of CAR target genes J Biol Chem 2003 May 9 278 19 17277 83 Epub 2003 Feb 27 PubMed PMID 12611900 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Maglich JM Parks DJ Moore LB Collins JL Goodwin B Billin AN Stoltz CA Kliewer SA Lambert MH Willson TM Moore JT Identification of a novel human constitutive androstane receptor CAR agonist and its use in the identification of CAR target genes J Biol Chem 2003 May 9 278 19 17277 83 Epub 2003 Feb 27 PubMed PMID 12611900 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A

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4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

<u>Date of Assay Document Creation:</u>

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hCAR_Antagonist

Assay Title: Novascreen human unspecified nuclear receptor subfamily 1, group I, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hCAR_Antagonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hCAR_Antagonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NR1I3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hCAR_Antagonist, is one of 1 assay component(s) measured or calculated from the NVS_NR_hCAR_Antagonist assay. It is designed to make measurements of fluorescent resonance energy transfer, a form of binding reporter, as detected with fluorescent resonance energy transfer signals by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hCAR_Antagonist is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.683
Neutral control median absolute deviation, by plate:	0.029652
Positive control well median response value, by plate:	0.187
Positive control well median absolute deviation, by plate:	0.007413
Negative control well median, by plate:	104.484605087015
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-55
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-55.98
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.09255137925133
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Maglich JM, Parks DJ, Moore LB, Collins JL, Goodwin B, Billin AN, Stoltz CA, Kliewer SA, Lambert MH, Willson TM, Moore JT. Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. J Biol Chem. 2003 May 9;278(19):17277-83. Epub 2003 Feb 27. PubMed PMID: 12611900.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Maglich JM Parks DJ Moore LB Collins JL Goodwin B Billin AN Stoltz CA Kliewer SA Lambert MH Willson TM Moore JT Identification of a novel human constitutive androstane receptor CAR agonist and its use in the identification of CAR target genes J Biol Chem 2003 May 9 278 19 17277 83 Epub 2003 Feb 27 PubMed PMID 12611900 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Maglich JM Parks DJ Moore LB Collins JL Goodwin B Billin AN Stoltz CA Kliewer SA Lambert MH Willson TM Moore JT Identification of a novel human constitutive androstane receptor CAR agonist and its use in the identification of CAR target genes J Biol Chem 2003 May 9 278 19 17277 83 Epub 2003 Feb 27 PubMed PMID 12611900 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A

Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

<u>Contact Information:</u>

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

<u>Date of Assay Document Creation:</u>

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hGR

Assay Title: Novascreen human unspecified nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hGR was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hGR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NR3C1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hGR, is one of 1 assay component(s) measured or calculated from the NVS_NR_hGR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hGR is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 16 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5654
Neutral control median absolute deviation, by plate:	243.146
Positive control well median response value, by plate:	219
Positive control well median absolute deviation, by plate:	17.7912
Negative control well median, by plate:	133.75573863279
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.57
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.82356679827413
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.9414007896448

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Han D, Ogita K, Kashiwai K, Narita S, Yoneda Y. Binding of [3H]triamcinolone acetonide to glucocorticoid receptors in brain cytosol fractions of rats with intact adrenals. Neurochem Int. 1994 Apr;24(4):339-48. PubMed PMID: 8061598.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Han D Ogita K Kashiwai K Narita S Yoneda Y Binding of 3H triamcinolone acetonide to glucocorticoid receptors in brain cytosol fractions of rats with intact adrenals Neurochem Int 1994 Apr 24 4 339 48 PubMed PMID 8061598 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hPR

Assay Title: Novascreen human unspecified progesterone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hPR was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hPR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene PGR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hPR, is one of 1 assay component(s) measured or calculated from the NVS_NR_hPR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hPR is a biochemical, single-readout assay that uses extracted gene-proteins from T47D in a cell-free assay.Measurements were taken 18 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5954.02
Neutral control median absolute deviation, by plate:	212.72
Positive control well median response value, by plate:	499.355
Positive control well median absolute deviation, by plate:	51.1497
Negative control well median, by plate:	132.007575757576
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.84
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.26
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.97958388964505
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.8775033378703

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Traish AM, MÃ¼ller RE, Wotiz HH. Binding of 7 alpha, 17 alpha-dimethyl-19-nortestosterone (mibolerone) to androgen and progesterone receptors in human and animal tissues. Endocrinology. 1986 Apr;118(4):1327-33. PubMed PMID: 2419120.|Haji M, Kato KI, Nawata H, Ibayashi H. Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat. Brain Res. 1981 Jan 12;204(2):373-86. PubMed PMID: 6780133.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Traish AM M ller RE Wotiz HH Binding of 7 alpha 17 alpha dimethyl 19 nortestosterone mibolerone to androgen and progesterone receptors in human and animal tissues Endocrinology 1986 Apr 118 4 1327 33 PubMed PMID 2419120 Haji M Kato KI Nawata H Ibayashi H Age related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat Brain Res 1981 Jan 12 204 2 373 86 PubMed PMID 6780133 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hPXR

Assay Title: Novascreen human unspecified nuclear receptor subfamily 1, group I, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hPXR was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hPXR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene NR1I2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hPXR, is one of 1 assay component(s) measured or calculated from the NVS_NR_hPXR assay. It is designed to make measurements of fluorescent resonance energy transfer, a form of binding reporter, as detected with fluorescent resonance energy transfer signals by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hPXR is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.216
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	0.08
Positive control well median absolute deviation, by plate:	0.0044478
Negative control well median, by plate:	137.5
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-20.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.36
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.42964285714285
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.5778571428571

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Rosenfeld JM, Vargas R Jr, Xie W, Evans RM. Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. Mol Endocrinol. 2003 Jul;17(7):1268-82. Epub 2003 Mar 27. PubMed PMID: 12663745|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Rosenfeld JM Vargas R Jr Xie W Evans RM Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor Mol Endocrinol 2003 Jul 17 7 1268 82 Epub 2003 Mar 27 PubMed PMID 12663745 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Rosenfeld JM Vargas R Jr Xie W Evans RM Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor Mol Endocrinol 2003 Jul 17 7 1268 82 Epub 2003 Mar 27 PubMed PMID 12663745 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hRAR_Antagonist

Assay Title: Novascreen human unspecified retinoic acid receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hRAR_Antagonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hRAR_Antagonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene RARA. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hRAR_Antagonist, is one of 1 assay component(s) measured or calculated from the NVS_NR_hRAR_Antagonist assay. It is designed to make measurements of fluorescent resonance energy transfer, a form of binding reporter, as detected with fluorescent resonance energy transfer signals by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hRAR_Antagonist is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.705
Neutral control median absolute deviation, by plate:	0.0203858
Positive control well median response value, by plate:	0.12
Positive control well median absolute deviation, by plate:	0.0044478
Negative control well median, by plate:	100.890868596882
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-26.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.395375271865
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.37225163119

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Chang Cy, Norris JD, GrÃ¸n H, Paige LA, Hamilton PT, Kenan DJ, Fowlkes D, McDonnell DP. Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: discovery of peptide antagonists of estrogen receptors alpha and beta. Mol Cell Biol. 1999 Dec;19(12):8226-39. PubMed PMID: 10567548; PubMed Central PMCID: PMC84907.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_hRARa_Agonist

Assay Title: Novascreen human unspecified retinoic acid receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_hRARa_Agonist was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_hRARa_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene RARA. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_NR_hRARa_Agonist, is one of 1 assay component(s) measured or calculated from the NVS_NR_hRARa_Agonist assay. It is designed to make measurements of fluorescent resonance energy transfer, a form of binding reporter, as detected with fluorescent resonance energy transfer signals by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_hRARa_Agonist is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 2 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.16
Neutral control median absolute deviation, by plate:	0.0059304
Positive control well median response value, by plate:	0.926
Positive control well median absolute deviation, by plate:	0.0415128
Negative control well median, by plate:	20424.8366013072
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	108.26
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	5.61
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.80315789473685
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	46.8189473684211

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Chang Cy, Norris JD, GrÃ¸n H, Paige LA, Hamilton PT, Kenan DJ, Fowlkes D, McDonnell DP. Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: discovery of peptide antagonists of estrogen receptors alpha and beta. Mol Cell Biol. 1999 Dec;19(12):8226-39. PubMed PMID: 10567548; PubMed Central PMCID: PMC84907.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_NR_rMR

Assay Title: Novascreen rat unspecified nuclear receptor subfamily 3, group C, member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_NR_rMR was analyzed into 1 assay endpoint. This assay endpoint, NVS_NR_rMR, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Nr3c2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "steroidal".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_NR_rMR, is one of 1 assay component(s) measured or calculated from the NVS_NR_rMR assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Lysate-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_NR_rMR is a biochemical, single-readout assay that uses extracted gene-proteins from Adrenalectomized rat brain supernatant in a tissue-based cell-free assay.Measurements were taken 24 hours after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1567.14
Neutral control median absolute deviation, by plate:	48.2142
Positive control well median response value, by plate:	337.775
Positive control well median absolute deviation, by plate:	22.1352
Negative control well median, by plate:	165.175030177617
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.81
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.56851906832463
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	39.4111144099478

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Beaumont K, Fanestil DD. Characterization of rat brain aldosterone receptors reveals high affinity for corticosterone. Endocrinology. 1983 Dec;113(6):2043-51. PubMed PMID: 6227474.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Beaumont K Fanestil DD Characterization of rat brain aldosterone receptors reveals high affinity for corticosterone Endocrinology 1983 Dec 113 6 2043 51 PubMed PMID 6227474 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_OR_gSIGMA_NonSelective

Assay Title: Novascreen guinea pig unspecified sigma non-opioid intracellular receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_OR_gSIGMA_NonSelective was analyzed into 1 assay endpoint. This assay endpoint, NVS_OR_gSIGMA_NonSelective, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Sigmar1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "misc protein" intended target family, where the subfamily is "chaperone".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_OR_gSIGMA_NonSelective, is one of 1 assay component(s) measured or calculated from the NVS_OR_gSIGMA_NonSelective assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_OR_gSIGMA_NonSelective is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig brain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5251.96
Neutral control median absolute deviation, by plate:	172.234
Positive control well median response value, by plate:	846.6
Positive control well median absolute deviation, by plate:	34.8633
Negative control well median, by plate:	115.572318743292
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-22
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-26.15
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

provisional

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.53953676072849
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.2372205643709

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Karbon EW, Naper K, Pontecorvo MJ. [3H]DTG and [3H](+)-3-PPP label pharmacologically distinct sigma binding sites in guinea pig brain membranes. Eur J Pharmacol. 1991 Jan 25;193(1):21-7. PubMed PMID: 1675607.|Weber E, Sonders M, Quarum M, McLean S, Pou S, Keana JF. 1,3-Di(2-[5-3H]tolyl)guanidine: a selective ligand that labels sigma-type receptors for psychotomimetic opiates and antipsychotic drugs. Proc Natl Acad Sci U S A. 1986 Nov;83(22):8784-8. PubMed PMID: 2877462; PubMed Central PMCID: PMC387016.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Karbon EW Naper K Pontecorvo MJ 3H DTG and 3H 3 PPP label pharmacologically distinct sigma binding sites in guinea pig brain membranes Eur J Pharmacol 1991 Jan 25 193 1 21 7 PubMed PMID 1675607 Weber E Sonders M Quarum M McLean S Pou S Keana JF 1 3 Di 2 5 3H tolyl guanidine a selective ligand that labels sigma type receptors for psychotomimetic opiates and antipsychotic drugs Proc Natl Acad Sci U S A 1986 Nov 83 22 8784 8 PubMed PMID 2877462 PubMed Central PMCID PMC387016 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Karbon EW Naper K Pontecorvo MJ 3H DTG and 3H 3 PPP label pharmacologically distinct sigma binding sites in guinea pig brain membranes Eur J Pharmacol 1991 Jan 25 193 1 21 7 PubMed PMID 1675607 Weber E

Sonders M Quarum M McLean S Pou S Keana JF 1 3 Di 2 5 3H tolyl guanidine a selective ligand that labels sigma type receptors for psychotomimetic opiates and antipsychotic drugs Proc Natl Acad Sci U S A 1986 Nov 83 22 8784 8 PubMed PMID 2877462 PubMed Central PMCID PMC387016 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Karbon EW Naper K Pontecorvo MJ 3H DTG and 3H 3 PPP label pharmacologically distinct sigma binding sites in guinea pig brain membranes Eur J Pharmacol 1991 Jan 25 193 1 21 7 PubMed PMID 1675607 Weber E Sonders M Quarum M McLean S Pou S Keana JF 1 3 Di 2 5 3H tolyl guanidine a selective ligand that labels sigma type receptors for psychotomimetic opiates and antipsychotic drugs Proc Natl Acad Sci U S A 1986 Nov 83 22 8784 8 PubMed PMID 2877462 PubMed Central PMCID PMC387016 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_OR_hFKBP12

Assay Title: Novascreen human unspecified FK506 binding protein 1A, 12kDa assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_OR_hFKBP12 was analyzed into 1 assay endpoint. This assay endpoint, NVS_OR_hFKBP12, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene FKBP1A. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "misc protein" intended target family, where the subfamily is "chaperone".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_OR_hFKBP12, is one of 1 assay component(s) measured or calculated from the NVS_OR_hFKBP12 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_OR_hFKBP12 is a biochemical, single-readout assay that uses extracted gene-proteins from E. coli in a cell-free assay.Measurements were taken 0.75 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6819.58
Neutral control median absolute deviation, by plate:	149.646
Positive control well median response value, by plate:	1075.58
Positive control well median absolute deviation, by plate:	46.8279
Negative control well median, by plate:	97.1802618092132
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.75
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-25.02
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.62744109474353
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.7646465684612

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Asami M, Kuno T, Mukai H, Tanaka C. Detection of the FK506-FKBP-calcineurin complex by a simple binding assay. Biochem Biophys Res Commun. 1993 May 14;192(3):1388-94. PubMed PMID: 7685169.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_gDAT

Assay Title: Novascreen guinea pig unspecified solute carrier family 6 (neurotransmitter transporter, dopamine), member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_gDAT was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_gDAT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Slc6a3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "neurotransmitter transporter".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_TR_gDAT, is one of 1 assay component(s) measured or calculated from the NVS_TR_gDAT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_gDAT is a biochemical, single-readout assay that uses extracted gene-proteins from Guinea pig striatal membranes in a tissue-based cell-free assay.Measurements were taken 2 hours after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	6041.43
Neutral control median absolute deviation, by plate:	201.886
Positive control well median response value, by plate:	1299.8
Positive control well median absolute deviation, by plate:	76.6993
Negative control well median, by plate:	129.929671187664
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-19
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-23.19
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

model

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.70476238752447
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.2285743251468

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Madras BK, Spealman RD, Fahey MA, Neumeyer JL, Saha JK, Milius RA. Cocaine receptors labeled by [3H]2 beta-carbomethoxy-3 beta-(4-fluorophenyl)tropane. Mol Pharmacol. 1989 Oct;36(4):518-24. PubMed PMID: 2811854.|Javitch JA, Blaustein RO, Snyder SH. [3H]mazindol binding associated with neuronal dopamine and norepinephrine uptake sites. Mol Pharmacol. 1984 Jul;26(1):35-44. PubMed PMID: 6087116.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Madras BK Spealman RD Fahey MA Neumeyer JL Saha JK Milius RA Cocaine receptors labeled by 3H 2 beta carbomethoxy 3 beta 4 fluorophenyl tropane Mol Pharmacol 1989 Oct 36 4 518 24 PubMed PMID 2811854 Javitch JA Blaustein RO Snyder SH 3H mazindol binding associated with neuronal dopamine and norepinephrine uptake sites Mol Pharmacol 1984 Jul 26 1 35 44 PubMed PMID 6087116 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 |Madras BK Spealman RD Fahey MA Neumeyer JL Saha JK Milius RA Cocaine receptors labeled by 3H 2 beta carbomethoxy 3 beta 4 fluorophenyl tropane Mol Pharmacol 1989 Oct 36 4 518 24 PubMed PMID 2811854 Javitch JA Blaustein RO Snyder SH 3H mazindol binding associated with neuronal dopamine and norepinephrine uptake sites Mol Pharmacol 1984 Jul 26 1 35 44 PubMed PMID 6087116 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878

95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949 Madras BK Spealman RD Fahey MA Neumeyer JL Saha JK Milius RA Cocaine receptors labeled by 3H 2 beta carbomethoxy 3 beta 4 fluorophenyl tropane Mol Pharmacol 1989 Oct 36 4 518 24 PubMed PMID 2811854 Javitch JA Blaustein RO Snyder SH 3H mazindol binding associated with neuronal dopamine and norepinephrine uptake sites Mol Pharmacol 1984 Jul 26 1 35 44 PubMed PMID 6087116 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_hAdoT

Assay Title: Novascreen human unspecified solute carrier family 29 (equilibrative nucleoside transporter), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_hAdoT was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_hAdoT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene SLC29A1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "nucleoside transporter".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_TR_hAdoT, is one of 1 assay component(s) measured or calculated from the NVS_TR_hAdoT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_hAdoT is a biochemical, single-readout assay that uses extracted gene-proteins from U937 in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	8082
Neutral control median absolute deviation, by plate:	131.951
Positive control well median response value, by plate:	248
Positive control well median absolute deviation, by plate:	17.0499
Negative control well median, by plate:	103.884964682139
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.93
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-53
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-55.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.38114545454546
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Marangos PJ, Patel J, Clark-Rosenberg R, Martino AM. [3H]nitrobenzylthioinosine binding as a probe for the study of adenosine uptake sites in brain. J Neurochem. 1982 Jul;39(1):184-91. PubMed PMID: 7086410.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Marangos PJ Patel J Clark Rosenberg R Martino AM 3H nitrobenzylthioinosine binding as a probe for the study of adenosine uptake sites in brain J Neurochem 1982 Jul 39 1 184 91 PubMed PMID 7086410 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_hDAT

Assay Title: Novascreen human unspecified solute carrier family 6 (neurotransmitter transporter), member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_hDAT was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_hDAT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene SLC6A3. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "neurotransmitter transporter".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_TR_hDAT, is one of 1 assay component(s) measured or calculated from the NVS_TR_hDAT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_hDAT is a biochemical, single-readout assay that uses extracted gene-proteins in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	14970.2
Neutral control median absolute deviation, by plate:	724.621
Positive control well median response value, by plate:	1773
Positive control well median absolute deviation, by plate:	95.6277
Negative control well median, by plate:	114.992314665195
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.73
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.15
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.32006520794786
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Madras BK, Spealman RD, Fahey MA, Neumeyer JL, Saha JK, Milius RA. Cocaine receptors labeled by [3H]2 beta-carbomethoxy-3 beta-(4-fluorophenyl)tropane. Mol Pharmacol. 1989 Oct;36(4):518-24. PubMed PMID: 2811854.|Javitch JA, Blaustein RO, Snyder SH. [3H]mazindol binding associated with neuronal dopamine and norepinephrine uptake sites. Mol Pharmacol. 1984 Jul;26(1):35-44. PubMed PMID: 6087116.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Madras BK Spealman RD Fahey MA Neumeyer JL Saha JK Milius RA Cocaine receptors labeled by 3H 2 beta carbomethoxy 3 beta 4 fluorophenyl tropane Mol Pharmacol 1989 Oct 36 4 518 24 PubMed PMID 2811854 Javitch JA Blaustein RO Snyder SH 3H mazindol binding associated with neuronal dopamine and norepinephrine uptake sites Mol Pharmacol 1984 Jul 26 1 35 44 PubMed PMID 6087116 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_hNET

Assay Title: Novascreen human unspecified solute carrier family 6 (neurotransmitter transporter), member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_hNET was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_hNET, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene SLC6A2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "neurotransmitter transporter".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified cell-free

Basic Procedure:
NVS_TR_hNET, is one of 1 assay component(s) measured or calculated from the NVS_TR_hNET assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_hNET is a biochemical, single-readout assay that uses extracted gene-proteins from CHO in a cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	3274.5
Neutral control median absolute deviation, by plate:	170.499
Positive control well median response value, by plate:	171
Positive control well median absolute deviation, by plate:	19.2738
Negative control well median, by plate:	122.060535506403
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-17.5
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.05
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.32927765614309
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.9756659368585

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or

semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_hSERT

Assay Title: Novascreen human unspecified solute carrier family 6 (neurotransmitter transporter), member 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_hSERT was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_hSERT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene SLC6A4. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "neurotransmitter transporter".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_TR_hSERT, is one of 1 assay component(s) measured or calculated from the NVS_TR_hSERT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_hSERT is a biochemical, single-readout assay that uses extracted gene-proteins from Human platelet membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	2006
Neutral control median absolute deviation, by plate:	133.434
Positive control well median response value, by plate:	201
Positive control well median absolute deviation, by plate:	31.1346
Negative control well median, by plate:	103.854314002829
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.72
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.89
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.29277283549391
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.7566370129635

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

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4.1 Resources

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Brown NL, Sirugue O, Worcel M. The effects of some slow channel blocking drugs on high affinity serotonin uptake by rat brain synaptosomes. Eur J Pharmacol. 1986 Apr 9;123(1):161-5. PubMed PMID: 2940099.|D'Amato RJ, Largent BL, Snowman AM, Snyder SH. Selective labeling of serotonin uptake sites in rat brain by [3H]citalopram contrasted to labeling of multiple sites by [3H]imipramine. J Pharmacol Exp Ther. 1987 Jul;242(1):364-71. PubMed PMID: 3475452.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_rAdoT

Assay Title: Novascreen rat unspecified solute carrier family 29 (equilibrative nucleoside transporter), member 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_rAdoT was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_rAdoT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Slc29a1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "nucleoside transporter".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_TR_rAdoT, is one of 1 assay component(s) measured or calculated from the NVS_TR_rAdoT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_rAdoT is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5986.47
Neutral control median absolute deviation, by plate:	140.773
Positive control well median response value, by plate:	1560.85
Positive control well median absolute deviation, by plate:	34.9152
Negative control well median, by plate:	98.1535153585576
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.87
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-26
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-27.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.26
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.12974336241893
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Marangos PJ, Patel J, Clark-Rosenberg R, Martino AM. [3H]nitrobenzylthioinosine binding as a probe for the study of adenosine uptake sites in brain. J Neurochem. 1982 Jul;39(1):184-91. PubMed PMID: 7086410.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Marangos PJ Patel J Clark Rosenberg R Martino AM 3H nitrobenzylthioinosine binding as a probe for the study of adenosine uptake sites in brain J Neurochem 1982 Jul 39 1 184 91 PubMed PMID 7086410 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_rNET

Assay Title: Novascreen rat unspecified solute carrier family 6 (neurotransmitter transporter), member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_rNET was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_rNET, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Slc6a2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "neurotransmitter transporter".

1.2 Assay Definition

Assay Throughput:

48-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_TR_rNET, is one of 1 assay component(s) measured or calculated from the NVS_TR_rNET assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_rNET is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 48-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	4396.1
Neutral control median absolute deviation, by plate:	136.518
Positive control well median response value, by plate:	982.065
Positive control well median absolute deviation, by plate:	35.79
Negative control well median, by plate:	112.008033947156
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.83
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.25713321860448
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Raisman R, Sette M, Pimoule C, Briley M, Langer SZ. High-affinity [3H]desipramine binding in the peripheral and central nervous system: a specific site associated with the neuronal uptake of noradrenaline. Eur J Pharmacol. 1982 Mar 12;78(3):345-51. PubMed PMID: 7067730.|Langer SZ, Raisman R, Briley M. High-affinity [3H] DMI binding is associated with neuronal noradrenaline uptake in the periphery and the central nervous system. Eur J Pharmacol. 1981 Jul 10;72(4):423-4. PubMed PMID: 7274336.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Raisman R Sette M Pimoule C Briley M Langer SZ High affinity 3H desipramine binding in the peripheral and central nervous system a specific site associated with the neuronal uptake of noradrenaline Eur J Pharmacol 1982 Mar 12 78 3 345 51 PubMed PMID 7067730 Langer SZ Raisman R Briley M High affinity 3H DMI binding is associated with neuronal noradrenaline uptake in the periphery and the central nervous system Eur J Pharmacol 1981 Jul 10 72 4 423 4 PubMed PMID 7274336 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_rSERT

Assay Title: Novascreen rat unspecified solute carrier family 6 (neurotransmitter transporter), member 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_rSERT was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_rSERT, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Slc6a4. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "neurotransmitter transporter".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_TR_rSERT, is one of 1 assay component(s) measured or calculated from the NVS_TR_rSERT assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_rSERT is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain membranes in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	5066
Neutral control median absolute deviation, by plate:	203.116
Positive control well median response value, by plate:	413.5
Positive control well median absolute deviation, by plate:	52.6323
Negative control well median, by plate:	107.126122608356
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.81
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-22.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.66726989365841
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.0036193619505

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Brown NL, Sirugue O, Worcel M. The effects of some slow channel blocking drugs on high affinity serotonin uptake by rat brain synaptosomes. Eur J Pharmacol. 1986 Apr 9;123(1):161-5. PubMed PMID: 2940099.|D'Amato RJ, Largent BL, Snowman AM, Snyder SH. Selective labeling of serotonin uptake sites in rat brain by [3H]citalopram contrasted to labeling of multiple sites by [3H]imipramine. J Pharmacol Exp Ther. 1987 Jul;242(1):364-71. PubMed PMID: 3475452.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

NVS_TR_rVMAT2

Assay Title: Novascreen rat unspecified solute carrier family 18 (vesicular monoamine transporter), member 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component NVS_TR_rVMAT2 was analyzed into 1 assay endpoint. This assay endpoint, NVS_TR_rVMAT2, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, loss-of-signal activity can be used to understand changes in the binding as they relate to the gene Slc18a2. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "transporter" intended target family, where the subfamily is "vesicular transporter".

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

unspecified tissue-based cell-free

Basic Procedure:
NVS_TR_rVMAT2, is one of 1 assay component(s) measured or calculated from the NVS_TR_rVMAT2 assay. It is designed to make measurements of radioligand binding, a form of binding reporter, as detected with scintillation counting signals by Filter-based radiodetection technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

NVS_TR_rVMAT2 is a biochemical, single-readout assay that uses extracted gene-proteins from Rat forebrain in a tissue-based cell-free assay.Measurements were taken 1 hour after chemical dosing in a 96-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1218
Neutral control median absolute deviation, by plate:	41.498
Positive control well median response value, by plate:	364
Positive control well median absolute deviation, by plate:	18.2434
Negative control well median, by plate:	118.125960061444
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.72
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-19.53
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	8
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.0199999999999999 µM
Standard maximum concentration tested:	50.0000000000001 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.17451986116468
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.0471191669881

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

NovaScreen, part of PerkinElmer, a human and environmental health company, provides a large diverse suite of cell-free binding and biochemical assays.

References:

Near JA. [3H]Dihydrotetrabenazine binding to bovine striatal synaptic vesicles. Mol Pharmacol. 1986 Sep;30(3):252-7. PubMed PMID: 3748008.|Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. Chem Res Toxicol. 2013 Jun 17;26(6):878-95. doi: 10.1021/tx400021f. Epub 2013 May 16. PubMed PMID: 23611293; PubMed Central PMCID: PMC3685188.|Knudsen TB, Houck KA, Sipes NS, Singh AV, Judson RS, Martin MT, Weissman A, Kleinstreuer NC, Mortensen HM, Reif DM, Rabinowitz JR, Setzer RW, Richard AM, Dix DJ, Kavlock RJ. Activity profiles of 309 ToxCastâ„¢ chemicals evaluated across 292 biochemical targets. Toxicology. 2011 Mar 28;282(1-2):1-15. doi: 10.1016/j.tox.2010.12.010. Epub 2011 Jan 18. PubMed PMID: 21251949.|Near JA 3H Dihydrotetrabenazine binding to bovine striatal synaptic vesicles Mol Pharmacol 1986 Sep 30 3 252 7 PubMed PMID 3748008 Sipes NS Martin MT Kothiya P Reif DM Judson RS Richard AM Houck KA Dix DJ Kavlock RJ Knudsen TB Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays Chem Res Toxicol 2013 Jun 17 26 6 878 95 doi 10 1021 tx400021f Epub 2013 May 16 PubMed PMID 23611293 PubMed Central PMCID PMC3685188 Knudsen TB Houck KA Sipes NS Singh AV Judson RS Martin MT Weissman A Kleinstreuer NC Mortensen HM Reif DM Rabinowitz JR Setzer RW Richard AM Dix DJ Kavlock RJ Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets Toxicology 2011 Mar 28 282 1 2 1 15 doi 10 1016 j tox 2010 12 010 Epub 2011 Jan 18 PubMed PMID 21251949

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_Apoptosis_PARP1_1440_inh

Assay Title: Odyssey Thera human U-2 OS unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent U-2 OS cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_Apoptosis_PARP1_1440_sti

Assay Title: Odyssey Thera human U-2 OS unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent U-2 OS cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_ATR_Chk1Cdc25C_0480_inh

Assay Title: Odyssey Thera human HEK293T checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_ATR_Chk1Cdc25C_0480_sti

Assay Title: Odyssey Thera human HEK293T checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

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Assay Source Information:

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MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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OT_ATR_Chk1Cdc25C_1440_inh

Assay Title: Odyssey Thera human HEK293T checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

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Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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4.1 Resources

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_ATR_Chk1Cdc25C_1440_sti

Assay Title: Odyssey Thera human HEK293T checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

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OT_ATR_Chk1Cdc25CCPT_1440_inh

Assay Title: Odyssey Thera human HEK293T checkpoint kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Z' (median across all plates, using positive control wells):	unspecified
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3. Assay Endpoint Descriptions

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Basic Procedure:
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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_b2AR_b2ARbARR2_0090_inh

Assay Title: Odyssey Thera human HEK293T adrenoceptor beta 2, surface assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_b2AR_b2ARbARR2_0090_sti

Assay Title: Odyssey Thera human HEK293T adrenoceptor beta 2, surface assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

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DMSO, Dimethyl Sulfoxide

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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OT_b2AR_b2ARbARR2_0180_inh

Assay Title: Odyssey Thera human HEK293T adrenoceptor beta 2, surface assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Standard minimum concentration tested:	unspecified µM
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1.1 Overview

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unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
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Proprietary Elements:

Assay is non-proprietary.

Caveats:

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None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Positive control well median response value, by plate:	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_b2AR_b2ARbARR2INN_0240_inh

Assay Title: Odyssey Thera human HEK293T adrenoceptor beta 2, surface assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_b2AR_b2ARbARR2INN_0240_sti

Assay Title: Odyssey Thera human HEK293T adrenoceptor beta 2, surface assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CellCycle_Cdc2Cdc25C_0480_inh

Assay Title: Odyssey Thera human HEK293 cyclin-dependent kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CellCycle_Cdc2Cdc25C_0480_sti

Assay Title: Odyssey Thera human HEK293 cyclin-dependent kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CellCycle_Cdc2Cdc25C_1440_inh

Assay Title: Odyssey Thera human HEK293 cyclin-dependent kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

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MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CellCycle_Cdc2Cdc25C_1440_sti

Assay Title: Odyssey Thera human HEK293 cyclin-dependent kinase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

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CYP, Cytochrome P450s

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ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CXCR4_CXCR4bARR2_0090_inh

Assay Title: Odyssey Thera human HEK293T chemokine (C-X-C motif) receptor 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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4. Assay Documentation

4.1 Resources

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CXCR4_CXCR4bARR2_0090_sti

Assay Title: Odyssey Thera human HEK293T chemokine (C-X-C motif) receptor 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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GST, Glutathione S-Transferase

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CXCR4_CXCR4bARR2_0480_inh

Assay Title: Odyssey Thera human HEK293T chemokine (C-X-C motif) receptor 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_CXCR4_CXCR4bARR2_0480_sti

Assay Title: Odyssey Thera human HEK293T chemokine (C-X-C motif) receptor 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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4. Assay Documentation

4.1 Resources

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Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

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4.2 Abbreviations and Definitions

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SULT, Sulfotransferases

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Date of Assay Document Creation:

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OT_CXCR4_CXCR4bARR2CXCL12_0240_inh

Assay Title: Odyssey Thera human HEK293T chemokine (C-X-C motif) receptor 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Experimental System:

adherent HEK293T cell line

Basic Procedure:
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Proprietary Elements:

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Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
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CV (median across all plates):	unspecified

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Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_ERStress_GRP78_1440_inh

Assay Title: Odyssey Thera human HeLa heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_ERStress_GRP78_1440_sti

Assay Title: Odyssey Thera human HeLa heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_H2AX_gH2AX_1440_inh

Assay Title: Odyssey Thera human U-2 OS H2A histone family, member X assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent U-2 OS cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_H2AX_gH2AX_1440_sti

Assay Title: Odyssey Thera human U-2 OS H2A histone family, member X assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent U-2 OS cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_HEK293T_LDH_2880_inh

Assay Title: Odyssey Thera human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_HEK293T_LDH_2880_sti

Assay Title: Odyssey Thera human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_Hepatocyte_LDH_2880_inh

Assay Title: Odyssey Thera human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_Hepatocyte_LDH_2880_sti

Assay Title: Odyssey Thera human hepatocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent hepatocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_0240_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_0240_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_0480_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_0480_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_0960_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_0960_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_1440_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_EmptyReporter_1440_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_0240_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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OT_NegCtrl_Fluorescence_0240_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_0480_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_0480_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_0960_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_0960_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_1440_inh

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NegCtrl_Fluorescence_1440_sti

Assay Title: Odyssey Thera human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NFKb_p50p65_0480_inh

Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NFKb_p50p65_0480_sti

Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NFKb_p50p65_1440_inh

Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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ATCC, American Tissue Culture Collection

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Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
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Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

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Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Date of Assay Document Creation:

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OT_NFKb_p50p65TNFa_0090_inh

Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Positive control well median response value, by plate:	unspecified
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Biological Response:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NFKb_p50p65TNFa_0480_inh

Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NFKb_p50p65TNFa_0480_sti

Assay Title: Odyssey Thera human HEK293 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NURR1_NURR1RXRa_0480

Assay Title: Odyssey Thera human HEK293T retinoid X receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component OT_NURR1_NURR1RXRa_0480 was analyzed into 1 assay endpoint. This assay endpoint, OT_NURR1_NURR1RXRa_0480, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, measures of receptor for gain-of-signal activity can be used to understand the binding at the pathway-level as they relate to the gene RXRA. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
OT_NURR1_NURR1RXRa_0480, is one of 1 assay component(s) measured or calculated from the OT_NURR1_NURR1RXRa_0480 assay. It is designed to make measurements of protein fragment complementation, a form of binding reporter, as detected with fluorescence intensity signals by Protein-fragment Complementation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

OT_NURR1_NURR1RXRa_0480 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 8 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	59.4145
Neutral control median absolute deviation, by plate:	5.52565
Positive control well median response value, by plate:	169.66
Positive control well median absolute deviation, by plate:	12.8149
Negative control well median, by plate:	9916.49449794564
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.54
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	24.2
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	3.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.03945070950227
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.1972535475113

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_NURR1_NURR1RXRa_1440

Assay Title: Odyssey Thera human HEK293T retinoid X receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component OT_NURR1_NURR1RXRa_1440 was analyzed into 1 assay endpoint. This assay endpoint, OT_NURR1_NURR1RXRa_1440, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of binding reporter, measures of receptor for gain-of-signal activity can be used to understand the binding at the pathway-level as they relate to the gene RXRA. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the "nuclear receptor" intended target family, where the subfamily is "non-steroidal".

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
OT_NURR1_NURR1RXRa_1440, is one of 1 assay component(s) measured or calculated from the OT_NURR1_NURR1RXRa_1440 assay. It is designed to make measurements of protein fragment complementation, a form of binding reporter, as detected with fluorescence intensity signals by Protein-fragment Complementation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

OT_NURR1_NURR1RXRa_1440 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 384-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	58.5825
Neutral control median absolute deviation, by plate:	5.53899
Positive control well median response value, by plate:	155.971
Positive control well median absolute deviation, by plate:	11.7437
Negative control well median, by plate:	1925.72211833482
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.44
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	18.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	2.83
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.1

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	6
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.3 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.15653062910042
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.7826531455021

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_p38MAPK_Mnk1p38_0480_inh

Assay Title: Odyssey Thera human HEK293T mitogen-activated protein kinase 14 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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OT_p38MAPK_Mnk1p38_0480_sti

Assay Title: Odyssey Thera human HEK293T mitogen-activated protein kinase 14 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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Chemical Library Scope and Limitations:

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4.1 Resources

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OT_p38MAPK_Mnk1p38_1440_inh

Assay Title: Odyssey Thera human HEK293T mitogen-activated protein kinase 14 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_p53_Mdm2p53_0480_inh

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

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OT_p53_Mdm2p53_0480_sti

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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4. Assay Documentation

4.1 Resources

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Date of Assay Document Creation:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_p53_Mdm2p53_1440_inh

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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OT_p53_Mdm2p53_1440_sti

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
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3.1 Data Interpretation

Biological Response:

unspecified

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_p53_Pin1p53_0480_inh

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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OT_p53_Pin1p53_0480_sti

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1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_p53_Pin1p53_1440_inh

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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4. Assay Documentation

4.1 Resources

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Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
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3.1 Data Interpretation

Biological Response:

unspecified

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_p53_Pin1p53CPT_1440_inh

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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OT_p53_Pin1p53CPT_1440_sti

Assay Title: Odyssey Thera human HEK293T tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_PI3K_Akt1PDk1_0090_inh

Assay Title: Odyssey Thera human HEK293 v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

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4.2 Abbreviations and Definitions

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

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4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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OT_PI3K_Akt1PDk1_0090_sti

Assay Title: Odyssey Thera human HEK293 v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Assay Title: Odyssey Thera human HEK293 v-akt murine thymoma viral oncogene homolog 1 assay

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1.1 Overview

Assay Summary:

unspecified

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Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_PI3K_Akt1PDk1_0480_sti

Assay Title: Odyssey Thera human HEK293 v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_PI3K_Akt1PDk1_1440_inh

Assay Title: Odyssey Thera human HEK293 v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_PI3K_Akt1PDk1_1440_sti

Assay Title: Odyssey Thera human HEK293 v-akt murine thymoma viral oncogene homolog 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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4. Assay Documentation

4.1 Resources

Assay Source Information:

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_RhoRock_Limk2Cofilin1_0480_inh

Assay Title: Odyssey Thera human HEK293T LIM domain kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_RhoRock_Limk2Cofilin1_0480_sti

Assay Title: Odyssey Thera human HEK293T LIM domain kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_RhoRock_Limk2Cofilin1_1440_inh

Assay Title: Odyssey Thera human HEK293T LIM domain kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

References:

MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006 Jun;2(6):329-37. Epub 2006 May 7. PubMed PMID: 16680159.|Yu H, West M, Keon BH, Bilter GK, Owens S, Lamerdin J, Westwick JK. Measuring drug action in the cellular context using protein-fragment complementation assays. Assay Drug Dev Technol. 2003 Dec;1(6):811-22. PubMed PMID: 15090227.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

OT_RhoRock_Limk2Cofilin1_1440_sti

Assay Title: Odyssey Thera human HEK293T LIM domain kinase 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Odyssey Thera is a drug discovery company focusing on high-throughput screening with a particular focus on developing novel protein:protein interaction assays using protein-fragment complementation technology.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_C13Cystine_area_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_C13Cystine_area_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_C13Ornithine_area_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_C13Ornithine_area_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_Cystine_area_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_Cystine_area_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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STEMINA_H9_CystineISnorm_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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SSMD (median across all plates, using negative control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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MIE, Molecular Initiating Event

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_CystineISnorm_perc_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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STEMINA_H9_CystinelSnorm_perc_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Positive control well median response value, by plate:	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

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Assay Performance Measures:

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1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_ORNCYSSISnorm_Prediction_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_ORNCYSSISnorm_Prediction_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_ORNCYSSISnorm_RATIO_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_ORNCYSSISnorm_RATIO_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_Ornithine_area_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_Ornithine_area_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_OrnithineISnorm_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_OrnithineISnorm_perc_dn

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_OrnithineISnorm_perc_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_OrnithineISnorm_up

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cell cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

STEMINA_H9_Viability_Norm

Assay Title: Stemina Biomarker Discovery human H9 embryonic neural stem cells unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension H9 embryonic neural stem cells cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_ActivityScore

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tanguay_ZF_120hpf_ActivityScore

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
Tanguay_ZF_120hpf_ActivityScore

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.477595
Neutral control median absolute deviation, by plate:	2.10458
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.113198297966
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	2.54

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	32
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.2401450326604
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_AXIS_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.616468
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.266449567411
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.63537411808397
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_BRAI_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.346432
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.999501757396
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.10016804911582
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_CFIN_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.461788
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	104.843361150392
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.13485958486518
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_CIRC_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.0631235
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.812136093884
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.82599601046064
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_EYE_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.5406
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.889254334255
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.30241738279413
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_JAW_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.607228
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.298155391699
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.62920060840169
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_MORT_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	12.8466
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.657918190208
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	10.5442904469143
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	52.7214522345715

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_NC_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.0763186
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.090835281738
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.76910974413219
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_OTIC_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.357384
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.58136367292
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.97768058396892
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_PE_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.883044
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.515200143889
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.88514919770174
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_PFIN_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.436524
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.609532684971
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.18262089117193
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_PIG_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.230959
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.474518778736
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.93284296757538
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_SNOU_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.737194
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.608920820831
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.70483677373105
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_SOMI_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.294979
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.047910657724
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.97596700497027
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_SWIM_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.272784
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	103.647370762189
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.87649540155528
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_TR_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.337977
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.621645225233
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.25069751143067
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_TRUN_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.489256
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.4280628961
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.21501896749265
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Tanguay_ZF_120hpf_YSE_up

Assay Title: Tanguay Lab zebrafish dechorionated zebrafish embryo unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

96-well plate

Experimental System:

suspension dechorionated zebrafish embryo whole embryo

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tanguay Lab at the OSU Center for Genomic Research and Biocomputing

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.959895
Neutral control median absolute deviation, by plate:	0
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.89701272264
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.00599999999999995 µM
Standard maximum concentration tested:	59.9999999999995 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.19325001944697
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

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References:

Truong L, Reif DM, St Mary L, Geier MC, Truong HD, Tanguay RL. Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci. 2014 Jan;137(1):212-33. doi: 10.1093/toxsci/kft235. Epub 2013 Oct 17. PubMed PMID: 24136191; PubMed Central PMCID: PMC3871932.

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AChE_Inhibition_Colormetric

Assay Title: TOX21_AChE_Inhibition_Colormetric

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AChE_Inhibition_Fluor

Assay Title: Tox21/NCGC human SH-SY5Y acetylcholinesterase (Yt blood group) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent SH-SY5Y cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AhR_LUC_Agonist

Assay Title: Tox21/NCGC human HepG2 aryl hydrocarbon receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AhR_LUC_Agonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AhR_LUC_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AHR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is basic helix-loop-helix protein.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AhR_LUC_Agonist, is one of 1 assay component(s) measured or calculated from the Tox21_AhR_LUC_Agonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by CALUX luciferase quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AhR_LUC_Agonist is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0349872
Neutral control median absolute deviation, by plate:	1.78345
Positive control well median response value, by plate:	99.4683
Positive control well median absolute deviation, by plate:	20.8621
Negative control well median, by plate:	459.3164099308
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.29
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	55.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-2818.51
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-48.79

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.20983399992497
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

He G, Tsutsumi T, Zhao B, Baston DS, Zhao J, Heath-Pagliuso S, Denison MS. Third-generation Ah receptor-responsive luciferase reporter plasmids: amplification of dioxin-responsive elements dramatically increases CALUX bioassay sensitivity and responsiveness. Toxicol Sci. 2011 Oct;123(2):511-22. doi: 10.1093/toxsci/kfr189. Epub 2011 Jul 20. PubMed PMID: 21775728; PubMed Central PMCID: PMC3179681.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AhR_LUC_Agonist_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_AhR_LUC_Agonist_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AhR_LUC_Agonist_viability is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (fluorescent protein induction) detected with Luciferase-coupled ATP quantitation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AhR_LUC_Agonist is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.147317
Neutral control median absolute deviation, by plate:	7.39186
Positive control well median response value, by plate:	-18.3156
Positive control well median absolute deviation, by plate:	10.3675
Negative control well median, by plate:	117.6593896533
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	106.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-50.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.67224378936707
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.0334627362024

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

He G, Tsutsumi T, Zhao B, Baston DS, Zhao J, Heath-Pagliuso S, Denison MS. Third-generation Ah receptor-responsive luciferase reporter plasmids: amplification of dioxin-responsive elements dramatically increases CALUX bioassay sensitivity and responsiveness. Toxicol Sci. 2011 Oct;123(2):511-22. doi: 10.1093/toxsci/kfr189. Epub 2011 Jul 20. PubMed PMID: 21775728; PubMed Central PMCID: PMC3179681.

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AP1_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-2.51584
Neutral control median absolute deviation, by plate:	7.0174
Positive control well median response value, by plate:	-28.4865
Positive control well median absolute deviation, by plate:	4.10883
Negative control well median, by plate:	90.8907854353
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-3
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-3.55
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	9.4
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-2.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.57591984669775
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AP1_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.25216
Positive control well median response value, by plate:	98.4239
Positive control well median absolute deviation, by plate:	6.21163
Negative control well median, by plate:	271.83934547
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.56
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	11.99
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	12.56
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	1.16

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.55134017774949
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.3080410664969

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AP1_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human ME-180 jun proto-oncogene|FBJ murine osteosarcoma viral oncogene homolog|jun proto-oncogene assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.765208
Neutral control median absolute deviation, by plate:	6.39683
Positive control well median response value, by plate:	104.332
Positive control well median absolute deviation, by plate:	7.52008
Negative control well median, by plate:	172.533389766
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.59
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	10
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	16.09
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	30.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	1.88

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.7369041511147
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AP1_BLA_Agonist_viability

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-3.89461
Neutral control median absolute deviation, by plate:	6.11497
Positive control well median response value, by plate:	4.74072
Positive control well median absolute deviation, by plate:	7.84915
Negative control well median, by plate:	104.5902530408
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	1.41
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-0.51
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.11

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.76010222277627
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.5606133366576

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_AR_BLA_Agonist_ch1

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AR_BLA_Agonist_ch1 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.18436
Neutral control median absolute deviation, by plate:	6.27003
Positive control well median response value, by plate:	-8.8623
Positive control well median absolute deviation, by plate:	5.81123
Negative control well median, by plate:	86.2959958237
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	43.35
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-34.42

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.80662653064722
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.8397591838833

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_AR_BLA_Agonist_ch2

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AR_BLA_Agonist_ch2 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.132224
Neutral control median absolute deviation, by plate:	4.4975
Positive control well median response value, by plate:	99.4999
Positive control well median absolute deviation, by plate:	21.7141
Negative control well median, by plate:	1595.1870757558
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.21
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	22.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-754.77
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-33.77

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.12833566869616
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human HEK293T androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_BLA_Agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AR_BLA_Agonist_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_AR_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.19755
Neutral control median absolute deviation, by plate:	6.37385
Positive control well median response value, by plate:	99.3555
Positive control well median absolute deviation, by plate:	21.5352
Negative control well median, by plate:	790.6969982163
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.15
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	15.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-412
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.54

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.30270280445154
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.185602
Neutral control median absolute deviation, by plate:	7.39323
Positive control well median response value, by plate:	24.0749
Positive control well median absolute deviation, by plate:	4.79682
Negative control well median, by plate:	866.5685790301
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	3
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	3.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-126.06
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-40.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.67643118576489
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.0585871145893

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

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Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.453936
Neutral control median absolute deviation, by plate:	16.945
Positive control well median response value, by plate:	-100.45
Positive control well median absolute deviation, by plate:	3.96053
Negative control well median, by plate:	164.132473771
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.39
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-6.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	227.94
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-37.8

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.80014108229746
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	52.8008464937848

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_BLA_Antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AR. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AR_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_AR_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.188074
Neutral control median absolute deviation, by plate:	6.93076
Positive control well median response value, by plate:	-100.309
Positive control well median absolute deviation, by plate:	3.04344
Negative control well median, by plate:	151.2557679062
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.71
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.97
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	510.39
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-36.98

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.34874025461246
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.0924415276748

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_BLA_Antagonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_BLA_Antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AR_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_AR_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.186187
Neutral control median absolute deviation, by plate:	7.50627
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	125.0611616461
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-40.56

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.8431979898606
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.0591879391636

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Agonist

Assay Title: Tox21/NCGC human MDA-kb2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_LUC_MDAKB2_Agonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_LUC_MDAKB2_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
Tox21_AR_LUC_MDAKB2_Agonist, is one of 1 assay component(s) measured or calculated from the Tox21_AR_LUC_MDAkb2_Agonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_LUC_MDAkb2_Agonist is a cell-based, single-readout assay that uses MDA-kb2, a human breast cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.111173
Neutral control median absolute deviation, by plate:	3.34421
Positive control well median response value, by plate:	99.5078
Positive control well median absolute deviation, by plate:	7.91212
Negative control well median, by plate:	514.3526512743
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.66
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	29.73
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-893.63
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-29.54

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.00963885106217
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Agonist2

Assay Title: Tox21/NCGC human MDA-kb2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_LUC_MDAKB2_Agonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_LUC_MDAKB2_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	1.19148
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	7.51192
Negative control well median, by plate:	479.9432305434
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.74
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	83.93
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.76833537423426
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Agonist2_viability

Assay Title: Tox21/NCGC human MDA-kb2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.94662
Positive control well median response value, by plate:	-2.4317
Positive control well median absolute deviation, by plate:	3.20699
Negative control well median, by plate:	107.1388702664
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.62
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.8938989638513
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Antagonist

Assay Title: Tox21/NCGC human MDA-kb2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_LUC_MDAKB2_Antagonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_LUC_MDAKB2_Antagonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
Tox21_AR_LUC_MDAKB2_Antagonist, is one of 1 assay component(s) measured or calculated from the Tox21_AR_LUC_MDAkb2_Antagonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_LUC_MDAkb2_Antagonist is a cell-based, single-readout assay that uses MDA-kb2, a human breast cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.247384
Neutral control median absolute deviation, by plate:	8.70592
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	2.40283
Negative control well median, by plate:	114.080696626
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.66
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.43
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	403.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-38.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.98058550254545
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.8835130152727

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Antagonist_viability

Assay Title: Tox21/NCGC human MDA-kb2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_AR_LUC_MDAKB2_Antagonist_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
Tox21_AR_LUC_MDAKB2_Antagonist_viability is an assay readout measuring viability via receptor activity and designed using NA (NA) detected with Luciferase-coupled ATP quantitation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AR_LUC_MDAkb2_Antagonist is a cell-based, single-readout assay that uses MDA-kb2, a human breast cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.178282
Neutral control median absolute deviation, by plate:	7.01211
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	117.576731625
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-39.34

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.00006271074825
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Antagonist2

Assay Title: Tox21/NCGC human MDA-kb2 androgen receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AR_LUC_MDAKB2_Antagonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AR_LUC_MDAKB2_Antagonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene AR. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.67739
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.89624
Negative control well median, by plate:	129.6405054994
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.68
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.52
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.35443940025589
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.1266364015353

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AR_LUC_MDAKB2_Antagonist2_viability

Assay Title: Tox21/NCGC human MDA-kb2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_AR_LUC_MDAKB2_Antagonist_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MDA-kb2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.9202
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.88879839025
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.73744592492177
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ARE_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ARE_BLA_Agonist is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.167331
Neutral control median absolute deviation, by plate:	4.86488
Positive control well median response value, by plate:	-25.0439
Positive control well median absolute deviation, by plate:	3.75646
Negative control well median, by plate:	75.5165015622
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-5
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	144.62
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.6

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.85455403754395
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ARE_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ARE_BLA_Agonist is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.339448
Neutral control median absolute deviation, by plate:	9.60923
Positive control well median response value, by plate:	99.4715
Positive control well median absolute deviation, by plate:	18.4331
Negative control well median, by plate:	1350.3035890366
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.14
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	10.37
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-292.88
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.8

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.63532060098133
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.811923605888

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ARE_BLA_agonist_ratio

Assay Title: Tox21/NCGC human HepG2 nuclear factor, erythroid 2-like 2 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ARE_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ARE_BLA_agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene NFE2L2. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is basic leucine zipper.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_ARE_BLA_Agonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_ARE_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ARE_BLA_Agonist is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.159843
Neutral control median absolute deviation, by plate:	4.93182
Positive control well median response value, by plate:	99.845
Positive control well median absolute deviation, by plate:	5.6632
Negative control well median, by plate:	203.9522564716
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.68
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	20.29
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-616.9
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-27.29

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.50978290890691
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ARE_BLA_agonist_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ARE_BLA_agonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ARE_BLA_agonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_ARE_BLA_agonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_ARE_BLA_Agonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ARE_BLA_Agonist is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.208165
Neutral control median absolute deviation, by plate:	6.67429
Positive control well median response value, by plate:	-0.296456
Positive control well median absolute deviation, by plate:	12.3333
Negative control well median, by plate:	112.8096348394
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.01
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	1.22
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-31.13

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.93299441089742
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.5979664653845

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_Aromatase_Inhibition

Assay Title: Tox21/NCGC human MCF-7 cytochrome P450, family 19, subfamily A, polypeptide 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_Aromatase_Inhibition was analyzed into 1 assay endpoint. This assay endpoint, Tox21_Aromatase_Inhibition, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene CYP19A1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cyp intended target family, where the subfamily is steroidogenesis-related.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MCF-7 cell line

Basic Procedure:
Tox21_Aromatase_Inhibition, is one of 1 assay component(s) measured or calculated from the Tox21_Aromatase_Inhibition assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_Aromatase_Inhibition is a cell-based, single-readout assay that uses MCF-7, a human breast cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.161868
Neutral control median absolute deviation, by plate:	6.28549
Positive control well median response value, by plate:	-100.053
Positive control well median absolute deviation, by plate:	0.901763
Negative control well median, by plate:	128.6890817401
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	617.98
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-36.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.46655699988806
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.7993419993284

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Chen S, Zhou D, Hsin LY, Kanaya N, Wong C, Yip R, Sakamuru S, Xia M, Yuan YC, Witt K, Teng C. AroER tri-screen is a biologically relevant assay for endocrine disrupting chemicals modulating the activity of aromatase and/or the estrogen receptor. Toxicol Sci. 2014 May;139(1):198-209. doi: 10.1093/toxsci/kfu023. Epub 2014 Feb 4. PubMed PMID: 24496634; PubMed Central PMCID: PMC4038786.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_Aromatase_Inhibition_viability

Assay Title: Tox21/NCGC human MCF-7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_Aromatase_Inhibition_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent MCF-7 cell line

Basic Procedure:
Tox21_Aromatase_Inhibition_viability is an assay readout measuring viability via receptor activity and designed using NA (NA) detected with Luciferase-coupled ATP quantitation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_Aromatase_Inhibition is a cell-based, single-readout assay that uses MCF-7, a human breast cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.179104
Neutral control median absolute deviation, by plate:	7.07455
Positive control well median response value, by plate:	-100.076
Positive control well median absolute deviation, by plate:	1.19671
Negative control well median, by plate:	140.041453037
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.75
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	559.96
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-41.97

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is

considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.60495203182658
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.6297121909595

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Chen S, Zhou D, Hsin LY, Kanaya N, Wong C, Yip R, Sakamuru S, Xia M, Yuan YC, Witt K, Teng C. AroER tri-screen is a biologically relevant assay for endocrine disrupting chemicals modulating the activity of aromatase and/or the estrogen receptor. Toxicol Sci. 2014 May;139(1):198-209. doi: 10.1093/toxsci/kfu023. Epub 2014 Feb 4. PubMed PMID: 24496634; PubMed Central PMCID: PMC4038786.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEK293_Cell_blue

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEK293_Cell_blue was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEK293_Cell_blue, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AutoFluor_HEK293_Cell_blue, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEK293 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEK293 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00317391
Neutral control median absolute deviation, by plate:	0.353355
Positive control well median response value, by plate:	12.1552
Positive control well median absolute deviation, by plate:	16.5598
Negative control well median, by plate:	7155.34198254735
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	30.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-2587.33
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-107.86

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.31029923829114
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEK293_Cell_green

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEK293_Cell_green was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEK293_Cell_green, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AutoFluor_HEK293_Cell_green, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEK293 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEK293 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00346571
Neutral control median absolute deviation, by plate:	0.559927
Positive control well median response value, by plate:	42.5568
Positive control well median absolute deviation, by plate:	58.7868
Negative control well median, by plate:	16531.5482283421
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	72.39
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-8157.93
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-149.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.18851312684946
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEK293_Cell_red

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEK293_Cell_red was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEK293_Cell_red, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AutoFluor_HEK293_Cell_red, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEK293 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEK293 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0048462
Neutral control median absolute deviation, by plate:	0.779281
Positive control well median response value, by plate:	751.847
Positive control well median absolute deviation, by plate:	983.326
Negative control well median, by plate:	2012.502263348
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	849.02
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-93142.16
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-117.24

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.67242503337177
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEK293_Media_blue

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEK293_Media_blue was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEK293_Media_blue, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AutoFluor_HEK293_Media_blue, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEK293 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEK293 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00389618
Neutral control median absolute deviation, by plate:	0.468013
Positive control well median response value, by plate:	14.7918
Positive control well median absolute deviation, by plate:	20.014
Negative control well median, by plate:	6886.15225730235
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	29.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-3456.91
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-111.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.25632367548543
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEK293_Media_green

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEK293_Media_green was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEK293_Media_green, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AutoFluor_HEK293_Media_green, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEK293 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEK293 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00403418
Neutral control median absolute deviation, by plate:	0.604782
Positive control well median response value, by plate:	41.0057
Positive control well median absolute deviation, by plate:	56.5195
Negative control well median, by plate:	16500.2880520593
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	66.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-9474.39
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-140.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.18493532520267
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEK293_Media_red

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEK293_Media_red was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEK293_Media_red, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_AutoFluor_HEK293_Media_red, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEK293 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEK293 is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00569435
Neutral control median absolute deviation, by plate:	0.593414
Positive control well median response value, by plate:	746.786
Positive control well median absolute deviation, by plate:	977.061
Negative control well median, by plate:	1961.3730151378
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	1243.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-129092.77
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-101.51

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.59991279052362
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEPG2_Cell_blue

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEPG2_Cell_blue was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEPG2_Cell_blue, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AutoFluor_HEPG2_Cell_blue, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEPG2 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEPG2 is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00440108
Neutral control median absolute deviation, by plate:	0.363061
Positive control well median response value, by plate:	0.068045
Positive control well median absolute deviation, by plate:	0.394532
Negative control well median, by plate:	8203.14631137665
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-9.88
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-79.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.24429501062085
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEPG2_Cell_green

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEPG2_Cell_green was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEPG2_Cell_green, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AutoFluor_HEPG2_Cell_green, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEPG2 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEPG2 is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0036838
Neutral control median absolute deviation, by plate:	0.446664
Positive control well median response value, by plate:	1643.83
Positive control well median absolute deviation, by plate:	2304.91
Negative control well median, by plate:	42693.6858588475
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	3860.53
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-481955.68
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-103

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.22809256670823
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEPG2_Cell_red

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEPG2_Cell_red was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEPG2_Cell_red, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AutoFluor_HEPG2_Cell_red, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEPG2 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEPG2 is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0059988
Neutral control median absolute deviation, by plate:	0.532024
Positive control well median response value, by plate:	0.234147
Positive control well median absolute deviation, by plate:	0.813944
Negative control well median, by plate:	1977.8357273304
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.38
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-30.01
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-97.22

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.59044409112297
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEPG2_Media_blue

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEPG2_Media_blue was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEPG2_Media_blue, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AutoFluor_HEPG2_Media_blue, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEPG2 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEPG2 is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00316376
Neutral control median absolute deviation, by plate:	0.406149
Positive control well median response value, by plate:	0.0468807
Positive control well median absolute deviation, by plate:	0.359531
Negative control well median, by plate:	7763.4821563827
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.11
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-8.79
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-125.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.21474193347348
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_AutoFluor_HEPG2_Media_green

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEPG2_Media_green was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEPG2_Media_green, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AutoFluor_HEPG2_Media_green, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEPG2 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEPG2 is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00325027
Neutral control median absolute deviation, by plate:	0.437413
Positive control well median response value, by plate:	1692.19
Positive control well median absolute deviation, by plate:	2383.13
Negative control well median, by plate:	40717.1088834215
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	3733.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-508814.03
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-110.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.20643743186052
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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TOX21_AutoFluor_HEPG2_Media_red

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_AutoFluor_HEPG2_Media_red was analyzed into 1 assay endpoint. This assay endpoint, Tox21_AutoFluor_HEPG2_Media_red, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of background reporter, gain-of-signal activity can be used to understand changes in the background control. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a background control function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the background measurement intended target family, where the subfamily is artifact fluorescence.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_AutoFluor_HEPG2_Media_red, is one of 6 assay component(s) measured or calculated from the Tox21_AutoFluor_HEPG2 assay. It is designed to make measurements of artifacts, a form of background reporter, as detected with fluorescence intensity signals by Autofluorescence technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_AutoFluor_HEPG2 is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.00698617
Neutral control median absolute deviation, by plate:	0.476334
Positive control well median response value, by plate:	0.0288514
Positive control well median absolute deviation, by plate:	0.681299
Negative control well median, by plate:	2030.91773114375
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-3.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-69.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	5
Target (nominal) number of replicates:	2
Standard minimum concentration tested:	0.1 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.41174604566193
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CAR_Agonist

Assay Title: Tox21/NCGC human HepG2 nuclear receptor subfamily 1, group I, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	1.71463
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	8.4029
Negative control well median, by plate:	245.69626548
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.7
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	58.32
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.83822208889671
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CAR_Agonist_viabillity

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.72518
Positive control well median response value, by plate:	6.7291
Positive control well median absolute deviation, by plate:	5.92989
Negative control well median, by plate:	114.4694579562
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.77710640921686
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CAR_Antagonist

Assay Title: Tox21/NCGC human HepG2 nuclear receptor subfamily 1, group I, member 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	10.8986
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.60279
Negative control well median, by plate:	155.7424826652
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.62
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-9.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.52508953720649
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.1505372232389

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CAR_Antagonist_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.05582
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	114.0845280692
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.40485484020376
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CASP3_CHO

Assay Title: TOX21_CASP3_CHO

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CASP3_CHO_viability

Assay Title: TOX21_CASP3_CHO_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CASP3_HEPG2

Assay Title: TOX21_CASP3_HEPG2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_CASP3_HEPG2_viability

Assay Title: TOX21_CASP3_HEPG2_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_DT40

Assay Title: Tox21/NCGC chicken DT40 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

suspension DT40 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.221407
Neutral control median absolute deviation, by plate:	6.9202
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	117.3515367509
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-22.79

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.0001030208237
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.0006181249422

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Winding P, Berchtold MW. The chicken B cell line DT40: a novel tool for gene disruption experiments. J Immunol Methods. 2001 Mar 1;249(1-2):1-16. Review. PubMed PMID: 11226459.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_DT40_100

Assay Title: Tox21/NCGC chicken DT40 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

suspension DT40 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.219305
Neutral control median absolute deviation, by plate:	6.49115
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	122.1908332012
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-24.36

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.59489896137582
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.5693937682549

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Winding P, Berchtold MW. The chicken B cell line DT40: a novel tool for gene disruption experiments. J Immunol Methods. 2001 Mar 1;249(1-2):1-16. Review. PubMed PMID: 11226459.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_DT40_657

Assay Title: Tox21/NCGC chicken DT40 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

suspension DT40 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.36651
Neutral control median absolute deviation, by plate:	8.42427
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	131.00495481035
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-21.52

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.36700254498032
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.2020152698819

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Winding P, Berchtold MW. The chicken B cell line DT40: a novel tool for gene disruption experiments. J Immunol Methods. 2001 Mar 1;249(1-2):1-16. Review. PubMed PMID: 11226459.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ELG1_LUC_Agonist

Assay Title: Tox21/NCGC human HEK293T ATPase family, AAA domain containing 5 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ELG1_LUC_Agonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ELG1_LUC_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene ATAD5. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the hydrolase intended target family, where the subfamily is atpase.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ELG1_LUC_Agonist, is one of 1 assay component(s) measured or calculated from the Tox21_ELG1_LUC_Agonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ELG1_LUC_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0864639
Neutral control median absolute deviation, by plate:	3.32236
Positive control well median response value, by plate:	99.6933
Positive control well median absolute deviation, by plate:	7.17147
Negative control well median, by plate:	115.0674246167
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.68
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	30.02
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1151.48
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-36.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.69423222004311
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ELG1_LUC_Agonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_ELG1_LUC_Agonist_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ELG1_LUC_Agonist_viability is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (fluorescent protein induction) detected with Luciferase-coupled ATP quantitation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ELG1_LUC_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.221698
Neutral control median absolute deviation, by plate:	8.19932
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	133.2092111106
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-35.41

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.38192653679485
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.2915592207691

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_ERa_BLA_Agonist_ch1

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ERa_BLA_Agonist_ch1 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.165857
Neutral control median absolute deviation, by plate:	5.51197
Positive control well median response value, by plate:	-33.7506
Positive control well median absolute deviation, by plate:	4.54625
Negative control well median, by plate:	90.152074637
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.14
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-6.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	204.34
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-30.71

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.92546209770346
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_ERa_BLA_Agonist_ch2

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ERa_BLA_Agonist_ch2 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0483974
Neutral control median absolute deviation, by plate:	1.58203
Positive control well median response value, by plate:	99.5603
Positive control well median absolute deviation, by plate:	14.6324
Negative control well median, by plate:	543.9150990534
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.51
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	62.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-2059.7
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.08

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.8158906126854
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human HEK293T estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ERa_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ERa_BLA_Agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene ESR1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ERa_BLA_Agonist_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_ERa_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0350631
Neutral control median absolute deviation, by plate:	1.12266
Positive control well median response value, by plate:	99.1775
Positive control well median absolute deviation, by plate:	20.8126
Negative control well median, by plate:	245.8817599947
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.33
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	87.86
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-2485.43
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-27.68

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.72596520807783
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.303577
Neutral control median absolute deviation, by plate:	9.91507
Positive control well median response value, by plate:	25.2348
Positive control well median absolute deviation, by plate:	9.98886
Negative control well median, by plate:	1498.9276501314
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	3.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-101.21
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-35.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.00060219534206
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.0036131720524

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.613128
Neutral control median absolute deviation, by plate:	20.0065
Positive control well median response value, by plate:	-100.071
Positive control well median absolute deviation, by plate:	2.72328
Negative control well median, by plate:	146.2282474827
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.3
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.96
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	163.48
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-33.83

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.48940110253932
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.9364066152359

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ERa_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ERa_BLA_Antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene ESR1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ERa_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_ERa_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.860952
Neutral control median absolute deviation, by plate:	19.3306
Positive control well median response value, by plate:	-100.09
Positive control well median absolute deviation, by plate:	2.9584
Negative control well median, by plate:	146.0528463348
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.31
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-5.13
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	115.14
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-21.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.3294568553358
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_BLA_Antagonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ERa_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ERa_BLA_Antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_ERa_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_ERa_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.273488
Neutral control median absolute deviation, by plate:	8.82643
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	112.2318525715
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-34.42

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.48187413715296
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.8912448229178

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Agonist

Assay Title: Tox21/NCGC human BG1 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ERa_LUC_BG1_Agonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ERa_LUC_BG1_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene ESR1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent BG1 cell line

Basic Procedure:
Tox21_ERa_LUC_BG1_Agonist, is one of 1 assay component(s) measured or calculated from the Tox21_ERa_LUC_BG1_Agonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_LUC_BG1_Agonist is a cell-based, single-readout assay that uses BG1, a human ovary cell line, with measurements taken at 48 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.179655
Neutral control median absolute deviation, by plate:	6.87099
Positive control well median response value, by plate:	99.5786
Positive control well median absolute deviation, by plate:	8.91392
Negative control well median, by plate:	361.7581615145
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.53
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	14.55
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-548.47
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-39.74

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.77515618820713
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Agonist2

Assay Title: TOX21_ERa_LUC_BG1_Agonist2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	0.928341
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	5.13873
Negative control well median, by plate:	186.2323077365
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.82
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	107.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.53381416222722
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Agonist2_viability

Assay Title: TOX21_ERa_LUC_BG1_Agonist2_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.4472
Positive control well median response value, by plate:	-1.39707
Positive control well median absolute deviation, by plate:	3.4964
Negative control well median, by plate:	109.6536465652
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.38
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.88022155075055
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Antagonist

Assay Title: Tox21/NCGC human BG1 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ERa_LUC_BG1_Antagonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ERa_LUC_BG1_Antagonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene ESR1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent BG1 cell line

Basic Procedure:
Tox21_ERa_LUC_BG1_Antagonist, is one of 1 assay component(s) measured or calculated from the Tox21_ERa_LUC_BG1_Antagonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_LUC_BG1_Antagonist is a cell-based, single-readout assay that uses BG1, a human ovary cell line, with measurements taken at 48 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.216491
Neutral control median absolute deviation, by plate:	7.32465
Positive control well median response value, by plate:	-100.054
Positive control well median absolute deviation, by plate:	0.902197
Negative control well median, by plate:	114.2019098922
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.75
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-13.64
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	462.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-35.2

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.08297841624554
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.4978704974732

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Antagonist_viability

Assay Title: Tox21/NCGC human BG1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_ERa_LUC_BG1_Antagonist_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent BG1 cell line

Basic Procedure:
Tox21_ERa_LUC_BG1_Antagonist_viability is an assay readout measuring viability via receptor activity and designed using NA (NA) detected with Luciferase-coupled ATP quantitation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ERa_LUC_BG1_Antagonist is a cell-based, single-readout assay that uses BG1, a human ovary cell line, with measurements taken at 48 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.178938
Neutral control median absolute deviation, by plate:	6.41507
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	118.938609297
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-35.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is

considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.09425324646842
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.5655194788105

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Antagonist2

Assay Title: Tox21/NCGC human BG1 estrogen receptor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent BG1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.81494
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.34372
Negative control well median, by plate:	108.5502840852
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.75
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-14.67
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.92655318948354
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.5593191369012

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERa_LUC_BG1_Antagonist2_viability

Assay Title: Tox21/NCGC human BG1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent BG1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	5.96314
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	102.9457678333
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.51263620085044
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	13.5647
Positive control well median response value, by plate:	-21.7278
Positive control well median absolute deviation, by plate:	13.1865
Negative control well median, by plate:	85.5376396531
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.44564025069353
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6738415041612

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.94225
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	25.2277
Negative control well median, by plate:	344.7381286696
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.15
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	33.99
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.20109945587492
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human HEK293T estrogen receptor 2 (ER beta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.33186
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	4.73698
Negative control well median, by plate:	135.3447033778
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.77
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	42.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.95171634960297
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Agonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.48866
Positive control well median response value, by plate:	8.40319
Positive control well median absolute deviation, by plate:	6.4145
Negative control well median, by plate:	105.1461473087
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	1.09
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.42496311979521
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.5497787187713

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	19.9023
Positive control well median response value, by plate:	19.0446
Positive control well median absolute deviation, by plate:	28.5887
Negative control well median, by plate:	332.5582131394
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.96
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.85686199310144
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.1411719586086

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	20.2223
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	5.87534
Negative control well median, by plate:	120.2446640233
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.22
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.09705587907717
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	36.582335274463

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T estrogen receptor 2 (ER beta) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	12.416
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	7.82749
Negative control well median, by plate:	135.4826777599
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.39
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.07
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.04208970819059
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.2525382491435

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERb_BLA_Antagonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.56687
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	100.185301648
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.51166013525229
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.0699608115137

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERR_Agonist

Assay Title: Tox21/NCGC human ERR-HEK293T estrogen-related receptor alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ERR-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.69813
Positive control well median response value, by plate:	15.5434
Positive control well median absolute deviation, by plate:	113.713
Negative control well median, by plate:	232.2836374811
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	6.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.20522521502626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERR_Antagonist

Assay Title: Tox21/NCGC human ERR-HEK293T estrogen-related receptor alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ERR-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.2411
Positive control well median response value, by plate:	26.8971
Positive control well median absolute deviation, by plate:	186.31
Negative control well median, by plate:	146.6714821565
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	6.1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.52182515338437
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.1309509203062

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ERR_viability

Assay Title: Tox21/NCGC human ERR-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ERR-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.48405
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.3120827881
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.25399102610353
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ESRE_BLA_ch1

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ESRE_BLA is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-1.94818
Neutral control median absolute deviation, by plate:	6.00105
Positive control well median response value, by plate:	-19.3365
Positive control well median absolute deviation, by plate:	4.75227
Negative control well median, by plate:	84.0536666476
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	4.76
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.43157765511288
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.5894659306773

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ESRE_BLA_ch2

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ESRE_BLA is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.161075
Neutral control median absolute deviation, by plate:	3.08757
Positive control well median response value, by plate:	102.852
Positive control well median absolute deviation, by plate:	10.856
Negative control well median, by plate:	1155.1904956974
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.59
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	33.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	32.41
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	0.87

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.7867772375936
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ESRE_BLA_ratio

Assay Title: Tox21/NCGC human HeLa activating transcription factor 6 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ESRE_BLA_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ESRE_BLA_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene ATF6. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is basic leucine zipper.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_ESRE_BLA_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_ESRE_BLA assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ESRE_BLA is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	1.05787
Neutral control median absolute deviation, by plate:	2.27878
Positive control well median response value, by plate:	98.0652
Positive control well median absolute deviation, by plate:	6.0388
Negative control well median, by plate:	428.6760647912
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.74
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	42.91
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	52.48
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	1.23

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.43781423195858
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_ESRE_BLA_viability

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_ESRE_BLA_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_ESRE_BLA_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_ESRE_BLA_viability, is one of 2 assay component(s) measured or calculated from the Tox21_ESRE_BLA assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_ESRE_BLA is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-2.07365
Neutral control median absolute deviation, by plate:	10.2433
Positive control well median response value, by plate:	-10.1223
Positive control well median absolute deviation, by plate:	11.035
Negative control well median, by plate:	124.362259038
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.83
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	1.63
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.42270991093352
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	44.5362594656011

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.392715
Neutral control median absolute deviation, by plate:	10.6743
Positive control well median response value, by plate:	-10.7012
Positive control well median absolute deviation, by plate:	8.41673
Negative control well median, by plate:	77.2669012861
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	16.79
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-27.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.34743856550218
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	32.0846313930131

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0636639
Neutral control median absolute deviation, by plate:	1.78417
Positive control well median response value, by plate:	99.4513
Positive control well median absolute deviation, by plate:	17.6078
Negative control well median, by plate:	581.7869926264
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.41
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	55.54
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1560.34
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.82

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.08122204967777
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_agonist_ratio

Assay Title: Tox21/NCGC human HEK293T nuclear receptor subfamily 1, group H, member 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_FXR_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_FXR_BLA_agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene NR1H4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_FXR_BLA_Agonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_FXR_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0889598
Neutral control median absolute deviation, by plate:	2.63818
Positive control well median response value, by plate:	99.4895
Positive control well median absolute deviation, by plate:	16.5624
Negative control well median, by plate:	243.0860211219
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.43
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	37.49
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1026.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-27.69

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.72013393556396
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_agonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_FXR_BLA_agonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_FXR_BLA_agonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_FXR_BLA_agonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_FXR_BLA_Agonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.500728
Neutral control median absolute deviation, by plate:	14.5996
Positive control well median response value, by plate:	-46.4064
Positive control well median absolute deviation, by plate:	15.4012
Negative control well median, by plate:	107.922867595
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-3.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	86.95
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.51

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.9150153371503
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	53.4900920229018

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.229537
Neutral control median absolute deviation, by plate:	6.87078
Positive control well median response value, by plate:	74.371
Positive control well median absolute deviation, by plate:	16.1005
Negative control well median, by plate:	970.5520609729
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.03
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	11.68
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-305.56
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-27.61

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.49378019015214
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.9626811409128

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.463835
Neutral control median absolute deviation, by plate:	15.3424
Positive control well median response value, by plate:	-100.697
Positive control well median absolute deviation, by plate:	12.0809
Negative control well median, by plate:	150.624597144
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.19
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-6.56
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	219.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-34.55

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.68364982286526
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	40.1018989371916

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

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NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T nuclear receptor subfamily 1, group H, member 4 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_FXR_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_FXR_BLA_antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene NR1H4. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_FXR_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_FXR_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.126679
Neutral control median absolute deviation, by plate:	4.82858
Positive control well median response value, by plate:	-100.376
Positive control well median absolute deviation, by plate:	6.09431
Negative control well median, by plate:	120.3151192999
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.67
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-20.67
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	778.69
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-37.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.78938673282934
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_FXR_BLA_antagonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_FXR_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_FXR_BLA_antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_FXR_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_FXR_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_FXR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.448882
Neutral control median absolute deviation, by plate:	14.6912
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	135.8855425491
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-31.59

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.81854571998712
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	40.9112743199227

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_GR_BLA_Agonist_ch1

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_GR_BLA_Agonist_ch1 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.214885
Neutral control median absolute deviation, by plate:	6.64995
Positive control well median response value, by plate:	-26.9542
Positive control well median absolute deviation, by plate:	6.49237
Negative control well median, by plate:	89.4765400677
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-3
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-3.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	111.17
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.29

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.6408008634627
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.8448051807762

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_GR_BLA_Agonist_ch2

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_GR_BLA_Agonist_ch2 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.118237
Neutral control median absolute deviation, by plate:	4.02466
Positive control well median response value, by plate:	99.4543
Positive control well median absolute deviation, by plate:	18.3473
Negative control well median, by plate:	531.6284632539
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.31
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	24.67
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-840.51
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-33.48

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.11430328142173
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human HeLa nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_GR_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_GR_BLA_Agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene NR3C1. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_GR_BLA_Agonist_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_GR_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0486325
Neutral control median absolute deviation, by plate:	1.8814
Positive control well median response value, by plate:	99.7748
Positive control well median absolute deviation, by plate:	7.96203
Negative control well median, by plate:	221.5044154203
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.7
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	53.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1813.36
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.18637193543015
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Antagonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.11205
Neutral control median absolute deviation, by plate:	6.93995
Positive control well median response value, by plate:	10.1713
Positive control well median absolute deviation, by plate:	7.3304
Negative control well median, by plate:	368.1072982154
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	1.44
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-89.58
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-63.62

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.32315222400194
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.9389133440116

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Antagonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.352901
Neutral control median absolute deviation, by plate:	20.2897
Positive control well median response value, by plate:	-100.164
Positive control well median absolute deviation, by plate:	5.89187
Negative control well median, by plate:	177.659982738
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.21
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.92
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	283.87
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-60.21

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.30860168158384
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	49.851610089503

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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CYP, Cytochrome P450s

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Antagonist_ratio

Assay Title: Tox21/NCGC human HeLa nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_GR_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_GR_BLA_Antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene NR3C1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_GR_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_GR_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Antagonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.187316
Neutral control median absolute deviation, by plate:	11.7595
Positive control well median response value, by plate:	-100.134
Positive control well median absolute deviation, by plate:	4.58282
Negative control well median, by plate:	182.7184206387
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.51
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.51
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	442.24
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-50.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.34390470252021
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.0634282151213

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_GR_BLA_Antagonist_viability

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_GR_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_GR_BLA_Antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_GR_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_GR_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_GR_BLA_Antagonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.181925
Neutral control median absolute deviation, by plate:	10.1157
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	108.4845915523
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-63

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.48463712459766
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.907822747586

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_H2AX_HTRF_CHO_Agonist_ch1

Assay Title: Tox21/NCGC chinese hamster CHO-K1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent CHO-K1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-2.39371
Neutral control median absolute deviation, by plate:	4.21215
Positive control well median response value, by plate:	90.5874
Positive control well median absolute deviation, by plate:	8.98419
Negative control well median, by plate:	44.2818194206
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.56
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	22.31
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-37.64
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.68

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00199999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.26154259505393
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_H2AX_HTRF_CHO_Agonist_ch2

Assay Title: Tox21/NCGC chinese hamster CHO-K1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent CHO-K1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-1.37229
Neutral control median absolute deviation, by plate:	5.26245
Positive control well median response value, by plate:	-4.89583
Positive control well median absolute deviation, by plate:	4.89202
Negative control well median, by plate:	927.3885906601
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	2.98
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-3.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00199999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.30482924833509
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_H2AX_HTRF_CHO_Agonist_ratio

Assay Title: Tox21/NCGC chinese hamster CHO-K1 H2A histone family, member X assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent CHO-K1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-1.53113
Neutral control median absolute deviation, by plate:	2.76259
Positive control well median response value, by plate:	71.2353
Positive control well median absolute deviation, by plate:	9.89336
Negative control well median, by plate:	1033.1259198461
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.46
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	26.26
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-44.35
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.84

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00199999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.1538473582706
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_H2AX_HTRF_CHO_viability

Assay Title: Tox21/NCGC chinese hamster CHO-K1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent CHO-K1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	5.95511
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.33209
Negative control well median, by plate:	104.6831716029
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.78
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-16
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-16.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.00199999999999999 µM
Standard maximum concentration tested:	199.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.15763115046871
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HDAC_Inhibition

Assay Title: Tox21/NCGC human HCT-116 histone deacetylase 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT-116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.59146
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.3621915337
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

histone deacetylase 1

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.55464412909631
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HDAC_Inhibition_viability

Assay Title: Tox21/NCGC human HCT-116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT-116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.46731
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.4248173118
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.94086260138813
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HRE_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.37015
Positive control well median response value, by plate:	-25.1079
Positive control well median absolute deviation, by plate:	2.79429
Negative control well median, by plate:	93.489818524
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.14
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-5.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.28898316931438
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HRE_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	1.11455
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	5.52271
Negative control well median, by plate:	382.1435930392
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.8
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	89.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.54401886785631
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HRE_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human ME-180 hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	0.576497
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	5.88443
Negative control well median, by plate:	204.6147802674
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.81
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	173.46
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.3624938875215
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HRE_BLA_Agonist_viability

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	5.24174
Positive control well median response value, by plate:	6.53829
Positive control well median absolute deviation, by plate:	6.26614
Negative control well median, by plate:	105.3127596685
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	1.2
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.0508964104962
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HSE_BLA_agonist_ch1

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_HSE_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.190153
Neutral control median absolute deviation, by plate:	5.13905
Positive control well median response value, by plate:	-21.1376
Positive control well median absolute deviation, by plate:	4.38672
Negative control well median, by plate:	89.4045489078
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-3
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-3.99
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	107.65
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-26.69

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.14273554940163
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HSE_BLA_agonist_ch2

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_HSE_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0458803
Neutral control median absolute deviation, by plate:	1.47051
Positive control well median response value, by plate:	99.4223
Positive control well median absolute deviation, by plate:	16.4274
Negative control well median, by plate:	516.4475576801
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.46
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	67.74
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-2172.68
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-31.29

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.96226530123801
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HSE_BLA_agonist_ratio

Assay Title: Tox21/NCGC human HeLa heat shock transcription factor 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_HSE_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_HSE_BLA_agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene HSF1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is heat shock protein.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_HSE_BLA_Agonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_HSE_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_HSE_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0490156
Neutral control median absolute deviation, by plate:	1.41766
Positive control well median response value, by plate:	99.2546
Positive control well median absolute deviation, by plate:	19.1844
Negative control well median, by plate:	327.4458532447
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.37
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	5
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	69.47
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1548.2
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-19.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.91844270028357
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_HSE_BLA_agonist_viability

Assay Title: Tox21/NCGC human HeLa unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_HSE_BLA_agonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_HSE_BLA_agonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HeLa cell line

Basic Procedure:
Tox21_HSE_BLA_agonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_HSE_BLA_Agonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_HSE_BLA_Agonist is a cell-based, single-readout assay that uses HeLa, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.516915
Neutral control median absolute deviation, by plate:	14.6957
Positive control well median response value, by plate:	-11.8512
Positive control well median absolute deviation, by plate:	11.6011
Negative control well median, by plate:	111.8943787224
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.8
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	21.13
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-26.18

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.78768837934283
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	46.726130276057

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_LUC_Biochem

Assay Title: TOX21_LUC_Biochem

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent unspecified cell-free

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.56807
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	0.235424
Negative control well median, by plate:	28.554844728
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.89
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-28
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-28.03
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	100 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.92286677597546
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_MMP_fitc

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_MMP_fitc

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_MMP_fitc is an assay readout measuring signaling via mitochondrial depolarization and designed using viability reporter (mitochondrial membrane potential) detected with immunofluorescence.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_MMP (Mitochondrial Membrane Potential) is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.152406
Neutral control median absolute deviation, by plate:	4.89605
Positive control well median response value, by plate:	1.1664
Positive control well median absolute deviation, by plate:	4.66805
Negative control well median, by plate:	1167.9005233913
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-5.79
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-31.92

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is

considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.92187633838886
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Sakamuru S, Li X, Attene-Ramos MS, Huang R, Lu J, Shou L, Shen M, Tice RR, Austin CP, Xia M. Application of a homogenous membrane potential assay to assess mitochondrial function. Physiol Genomics. 2012 May 1;44(9):495-503. doi: 10.1152/physiolgenomics.00161.2011. Epub 2012 Mar 20. PubMed PMID: 22433785; PubMed Central PMCID: PMC3426425.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_MMP_ratio_down

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_MMP_ratio was analyzed into 2 assay endpoints. This assay endpoint, Tox21_MMP_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of 6 membrane potential reporter, gain-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell morphology intended target family, where the subfamily is organelle conformation.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_MMP_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_MitochondrialToxicity assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by homogenous mitochondrial membrane potential assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_MMP (Mitochondrial Membrane Potential) is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.297544
Neutral control median absolute deviation, by plate:	10.7262
Positive control well median response value, by plate:	-100.023
Positive control well median absolute deviation, by plate:	1.4066
Negative control well median, by plate:	139.0035132716
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.63
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-9.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	309.49
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.71

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.44391923052108
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.6635153831265

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Sakamuru S, Li X, Attene-Ramos MS, Huang R, Lu J, Shou L, Shen M, Tice RR, Austin CP, Xia M. Application of a homogenous membrane potential assay to assess mitochondrial function. Physiol Genomics. 2012 May 1;44(9):495-503. doi: 10.1152/physiolgenomics.00161.2011. Epub 2012 Mar 20. PubMed PMID: 22433785; PubMed Central PMCID: PMC3426425.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_MMP_ratio_up

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_MMP_ratio_up was analyzed into 2 assay endpoint. The assay endpoin, Tox21_MMP_ratio_up, was analyzed in thenegative fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of membrane potential reportloss-of-signal activity can be used to understand changes in the signaling. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a signaling function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell morphology intended target family, where the subfamily is organelle conformation.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_MMP_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_MitochondrialToxicity assay. It is designed to make measurements of dye binding, a form of membrane potential reporter, as detected with fluorescence intensity signals by homogenous mitochondrial membrane potential assay technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_MMP (Mitochondrial Membrane Potential) is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.297544
Neutral control median absolute deviation, by plate:	10.7262
Positive control well median response value, by plate:	-100.023
Positive control well median absolute deviation, by plate:	1.4066
Negative control well median, by plate:	4242.3944614981
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.63
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-9.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	309.49
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.71

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.44391923052108
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	38.6635153831265

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Sakamuru S, Li X, Attene-Ramos MS, Huang R, Lu J, Shou L, Shen M, Tice RR, Austin CP, Xia M. Application of a homogenous membrane potential assay to assess mitochondrial function. Physiol Genomics. 2012 May 1;44(9):495-503. doi: 10.1152/physiolgenomics.00161.2011. Epub 2012 Mar 20. PubMed PMID: 22433785; PubMed Central PMCID: PMC3426425.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_MMP_rhodamine

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_MMP_rhodamine

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_MMP_rhodamine is an assay readout measuring signaling via mitochondrial depolarization and designed using viability reporter (mitochondrial membrane potential) detected with immunofluorescence.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_MMP (Mitochondrial Membrane Potential) is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.345516
Neutral control median absolute deviation, by plate:	11.6795
Positive control well median response value, by plate:	-100.022
Positive control well median absolute deviation, by plate:	1.29791
Negative control well median, by plate:	133.2603396912
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.61
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.53
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	289.5
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-30.54

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.44040343087034
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	44.642420585222

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Sakamuru S, Li X, Attene-Ramos MS, Huang R, Lu J, Shou L, Shen M, Tice RR, Austin CP, Xia M. Application of a homogenous membrane potential assay to assess mitochondrial function. Physiol Genomics. 2012 May 1;44(9):495-503. doi: 10.1152/physiolgenomics.00161.2011. Epub 2012 Mar 20. PubMed PMID: 22433785; PubMed Central PMCID: PMC3426425.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_MMP_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_MMP_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_MitochondrialToxicity_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
Tox21_MMP_viability, is one of 2 assay component(s) measured or calculated from the Tox21_MitochondrialToxicity assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_MMP (Mitochondrial Membrane Potential) is a cell-based, single-readout assay that uses HepG2, a human liver cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.178004
Neutral control median absolute deviation, by plate:	6.88241
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	107.8164827642
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-38.97

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.32276785161468
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Sakamuru S, Li X, Attene-Ramos MS, Huang R, Lu J, Shou L, Shen M, Tice RR, Austin CP, Xia M. Application of a homogenous membrane potential assay to assess mitochondrial function. Physiol Genomics. 2012 May 1;44(9):495-503. doi: 10.1152/physiolgenomics.00161.2011. Epub 2012 Mar 20. PubMed PMID: 22433785; PubMed Central PMCID: PMC3426425.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_NFkB_BLA_agonist_ch1

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_NFkB_BLA_Agonist is a cell-based, single-readout assay that uses ME-180, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.562279
Neutral control median absolute deviation, by plate:	5.87569
Positive control well median response value, by plate:	-47.3411
Positive control well median absolute deviation, by plate:	2.97319
Negative control well median, by plate:	87.6709092609
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.38
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-7.06
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.87
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.03

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.27484652838546
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_NFkB_BLA_agonist_ch2

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_NFkB_BLA_Agonist is a cell-based, single-readout assay that uses ME-180, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.110656
Neutral control median absolute deviation, by plate:	0.782974
Positive control well median response value, by plate:	97.5458
Positive control well median absolute deviation, by plate:	5.57241
Negative control well median, by plate:	246.4159517129
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.71
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	117.86
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	22.99
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	1.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.52645165987974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_NFkB_BLA_agonist_ratio

Assay Title: Tox21/NCGC human ME-180 nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_NFkB_BLA_agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_NFkB_BLA_agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene NFKB1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is NF-kappa B.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
Tox21_NFkB_BLA_agonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_NFkB_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_NFkB_BLA_Agonist is a cell-based, single-readout assay that uses ME-180, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.0539391
Neutral control median absolute deviation, by plate:	0.428863
Positive control well median response value, by plate:	99.426
Positive control well median absolute deviation, by plate:	2.8862
Negative control well median, by plate:	101.2421995191
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.88
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	27
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	222.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	1.86
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	1.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.28322094145254
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_NFkB_BLA_agonist_viability

Assay Title: Tox21/NCGC human ME-180 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_NFkB_BLA_agonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_NFkB_BLA_agonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent ME-180 cell line

Basic Procedure:
Tox21_NFkB_BLA_agonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_NFkB_BLA_Agonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_NFkB_BLA_Agonist is a cell-based, single-readout assay that uses ME-180, a human cervix cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.662037
Neutral control median absolute deviation, by plate:	8.91641
Positive control well median response value, by plate:	1.48535
Positive control well median absolute deviation, by plate:	6.72125
Negative control well median, by plate:	109.6085606578
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	0.96
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-0.98

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.08995358155937
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.5397214893562

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p1_ch1

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p1 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.144075
Neutral control median absolute deviation, by plate:	4.95434
Positive control well median response value, by plate:	-24.9276
Positive control well median absolute deviation, by plate:	3.60989
Negative control well median, by plate:	90.4202862466
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.79
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	161.04
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-34.59

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.00976293237186
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p1_ch2

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p1 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.12794
Neutral control median absolute deviation, by plate:	4.20621
Positive control well median response value, by plate:	99.156
Positive control well median absolute deviation, by plate:	12.5148
Negative control well median, by plate:	612.5842184594
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.49
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	23.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-775.72
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.27

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.71156233629626
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p1_ratio

Assay Title: Tox21/NCGC human HCT116 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p1_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p1_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is tumor suppressor.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p1_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p1 assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p1 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.115903
Neutral control median absolute deviation, by plate:	3.35928
Positive control well median response value, by plate:	99.4264
Positive control well median absolute deviation, by plate:	7.62637
Negative control well median, by plate:	477.31101799275
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.67
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	29.52
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-758.67
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-25.4

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.19928273802848
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p1_viability

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p1_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p1_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p1_viability, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p1 assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p1 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.225487
Neutral control median absolute deviation, by plate:	8.77392
Positive control well median response value, by plate:	-18.1056
Positive control well median absolute deviation, by plate:	14.4636
Negative control well median, by plate:	110.6621880312
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.99
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	76.57
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-37.67

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.99210027956454
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	41.9526016773872

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p2_ch1

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p2 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.16145
Neutral control median absolute deviation, by plate:	6.13244
Positive control well median response value, by plate:	-33.342
Positive control well median absolute deviation, by plate:	4.00255
Negative control well median, by plate:	99.392784839
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.03
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-5.35
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	193.3
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-35.7

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.8966141715805
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p2_ch2

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p2 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.11003
Neutral control median absolute deviation, by plate:	3.73094
Positive control well median response value, by plate:	99.1803
Positive control well median absolute deviation, by plate:	11.4625
Negative control well median, by plate:	402.7601900026
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.53
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	26.58
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-903.61
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-33.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.60204778429955
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p2_ratio

Assay Title: Tox21/NCGC human HCT116 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p2_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p2_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is tumor suppressor.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p2_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p2 assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p2 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.100452
Neutral control median absolute deviation, by plate:	2.8003
Positive control well median response value, by plate:	99.6407
Positive control well median absolute deviation, by plate:	4.03633
Negative control well median, by plate:	418.6629949915
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.8
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	20
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	35.65
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-902.5
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-24.46

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.95929146331389
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p2_viability

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p2_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p2_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p2_viability, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p2 assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p2 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.197839
Neutral control median absolute deviation, by plate:	7.76247
Positive control well median response value, by plate:	-16.3517
Positive control well median absolute deviation, by plate:	10.6025
Negative control well median, by plate:	116.8362992469
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.93
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	95.09
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-36.89

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.8818195738939
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	35.2909174433634

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p3_ch1

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p3 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.170622
Neutral control median absolute deviation, by plate:	6.35463
Positive control well median response value, by plate:	-32.831
Positive control well median absolute deviation, by plate:	4.21483
Negative control well median, by plate:	93.7565427874
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.02
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-5.14
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	190.54
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-41.83

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.42349065166122
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.5409439099673

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p3_ch2

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p3 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.130819
Neutral control median absolute deviation, by plate:	3.86642
Positive control well median response value, by plate:	98.2249
Positive control well median absolute deviation, by plate:	16.0885
Negative control well median, by plate:	790.8886849386
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.37
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	25.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-747.18
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-30.54

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.46618228323455
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p3_ratio

Assay Title: Tox21/NCGC human HCT116 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p3_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p3_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is tumor suppressor.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p3_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p3 assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p3 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.180343
Neutral control median absolute deviation, by plate:	3.80792
Positive control well median response value, by plate:	99.5761
Positive control well median absolute deviation, by plate:	6.91929
Negative control well median, by plate:	424.06597902515
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.67
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	26.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-545.81
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-22.24

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.8227628069073
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p3_viability

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p3_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p3_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p3_viability, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p3 assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p3 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.172978
Neutral control median absolute deviation, by plate:	7.01394
Positive control well median response value, by plate:	-19.157
Positive control well median absolute deviation, by plate:	10.9002
Negative control well median, by plate:	108.3425340011
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	111.79
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-44.01

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.02345831309728
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	30.1407498785837

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p4_ch1

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p4 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.163904
Neutral control median absolute deviation, by plate:	5.31453
Positive control well median response value, by plate:	-21.7163
Positive control well median absolute deviation, by plate:	3.29662
Negative control well median, by plate:	94.6544807045
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-3
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.07
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	130.59
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.31

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.74402988187153
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p4_ch2

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p4 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0912328
Neutral control median absolute deviation, by plate:	2.65499
Positive control well median response value, by plate:	99.5411
Positive control well median absolute deviation, by plate:	6.76033
Negative control well median, by plate:	498.5916716067
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.72
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	37.31
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1089.58
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-29.7

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.34760150021231
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p4_ratio

Assay Title: Tox21/NCGC human HCT116 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p4_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p4_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is tumor suppressor.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p4_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p4 assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p4 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.101449
Neutral control median absolute deviation, by plate:	2.86874
Positive control well median response value, by plate:	99.6235
Positive control well median absolute deviation, by plate:	4.19409
Negative control well median, by plate:	742.7602047424
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.78
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	19
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	34.75
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-892.57
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-26.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.02079986237957
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p4_viability

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p4_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p4_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p4_viability, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p4 assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p4 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.150411
Neutral control median absolute deviation, by plate:	5.99681
Positive control well median response value, by plate:	-15.4661
Positive control well median absolute deviation, by plate:	9.00292
Negative control well median, by plate:	113.8236839124
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2.48
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	96.11
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-41.38

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.98870130064896
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.9322078038938

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p5_ch1

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p5 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.169262
Neutral control median absolute deviation, by plate:	5.65338
Positive control well median response value, by plate:	-24.7688
Positive control well median absolute deviation, by plate:	4.88191
Negative control well median, by plate:	92.198586179
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-3
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.33
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	143.3
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-33.02

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.90755393557242
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.4453236134345

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p5_ch2

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p5 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0835641
Neutral control median absolute deviation, by plate:	3.07421
Positive control well median response value, by plate:	99.2386
Positive control well median absolute deviation, by plate:	12.4665
Negative control well median, by plate:	953.7308269967
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.53
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	32.21
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1165.51
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-35.3

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.61374125094707
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p5_ratio

Assay Title: Tox21/NCGC human HCT116 tumor protein p53 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p5_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p5_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene TP53. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the dna binding intended target family, where the subfamily is tumor suppressor.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p5_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p5 assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p5 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0786782
Neutral control median absolute deviation, by plate:	2.63573
Positive control well median response value, by plate:	99.2788
Positive control well median absolute deviation, by plate:	9.7017
Negative control well median, by plate:	584.516285504
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.63
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	10
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	37.25
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-1178.5
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-32.19

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.35224934572532
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_p53_BLA_p5_viability

Assay Title: Tox21/NCGC human HCT116 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_p53_BLA_p5_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_p53_BLA_p5_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HCT116 cell line

Basic Procedure:
Tox21_p53_BLA_p5_viability, is one of 2 assay component(s) measured or calculated from the Tox21_p53_BLA_p5 assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_p53_BLA_p5 is a cell-based, single-readout assay that uses HCT116, a human intestinal cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.195172
Neutral control median absolute deviation, by plate:	9.06657
Positive control well median response value, by plate:	-6.98907
Positive control well median absolute deviation, by plate:	10.0069
Negative control well median, by plate:	108.1883782729
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.72
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	30.57
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-43.64

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.374398559381
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	44.246391356286

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PGC_ERR_Agonist

Assay Title: Tox21/NCGC human PGC/ERR HEK293T estrogen-related receptor alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PGC/ERR HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.93109
Positive control well median response value, by plate:	29.269
Positive control well median absolute deviation, by plate:	88.5931
Negative control well median, by plate:	258.5343299882
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	9.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.68036626577288
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PGC_ERR_Antagonist

Assay Title: Tox21/NCGC human PGC/ERR HEK293T estrogen-related receptor alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PGC/ERR HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	10.1417
Positive control well median response value, by plate:	97.2158
Positive control well median absolute deviation, by plate:	298.834
Negative control well median, by plate:	222.2708227753
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	9.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.55685104879492
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.3411062927695

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PGC_ERR_viability

Assay Title: Tox21/NCGC human PGC/ERR HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PGC/ERR HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.24871
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.9207865173
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.5904653717656
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.262563
Neutral control median absolute deviation, by plate:	7.31086
Positive control well median response value, by plate:	-15.4872
Positive control well median absolute deviation, by plate:	4.85446
Negative control well median, by plate:	70.2227177395
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	56.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-28.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.83926539780367
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	35.035592386822

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.116643
Neutral control median absolute deviation, by plate:	3.17917
Positive control well median response value, by plate:	99.512
Positive control well median absolute deviation, by plate:	13.642
Negative control well median, by plate:	1351.8496547173
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.49
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	31.38
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-847.61
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-26.75

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.08611861938907
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_agonist_ratio

Assay Title: Tox21/NCGC human HEK293T peroxisome proliferator-activated receptor delta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARd_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARd_BLA_agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene PPARD. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARd_BLA_Agonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_PPARd_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.14381
Neutral control median absolute deviation, by plate:	4.0637
Positive control well median response value, by plate:	99.7402
Positive control well median absolute deviation, by plate:	7.63246
Negative control well median, by plate:	485.6939314699
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.64
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	24.57
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-639.78
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-26.69

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.9158737089685
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

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Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_Agonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARd_BLA_Agonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARd_BLA_Agonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARd_BLA_Agonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_PPARd_BLA_Agonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.304094
Neutral control median absolute deviation, by plate:	8.12161
Positive control well median response value, by plate:	-3.72802
Positive control well median absolute deviation, by plate:	7.20734
Negative control well median, by plate:	111.3703385254
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.37
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	9.51
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-26.04

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.55736672264977
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.3442003358986

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.143662
Neutral control median absolute deviation, by plate:	8.91444
Positive control well median response value, by plate:	27.2796
Positive control well median absolute deviation, by plate:	12.0821
Negative control well median, by plate:	900.4584606494
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	3.16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-198.68
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-58.41

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.65547205229335
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.9328323137601

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.405406
Neutral control median absolute deviation, by plate:	21.8123
Positive control well median response value, by plate:	-100.196
Positive control well median absolute deviation, by plate:	4.69163
Negative control well median, by plate:	189.0145402528
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.21
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.59
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	246.91
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-52.66

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	9.79828722001836
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	58.7897233201102

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T peroxisome proliferator-activated receptor delta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARd_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARd_BLA_antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene PPARD. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARd_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_PPARd_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.135564
Neutral control median absolute deviation, by plate:	8.80287
Positive control well median response value, by plate:	-100.269
Positive control well median absolute deviation, by plate:	7.01266
Negative control well median, by plate:	161.2551010648
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.52
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.37
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	685.08
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-60.91

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.04814805441609
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	36.2888883264965

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

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Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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RTCA, Real-Time Cell Analysis

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARd_BLA_antagonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARd_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARd_BLA_antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARd_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_PPARd_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARd_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.228921
Neutral control median absolute deviation, by plate:	13.1331
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	106.1947038866
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-57.05

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.98630529346328
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	47.9178317607797

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_PPARg_BLA_Agonist_ch1

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARg_BLA_Agonist_ch1 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.201108
Neutral control median absolute deviation, by plate:	7.36831
Positive control well median response value, by plate:	-21.5043
Positive control well median absolute deviation, by plate:	5.3091
Negative control well median, by plate:	78.391875808
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	107.79
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-36.47

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.59827516904273
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.5896510142564

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_PPARg_BLA_Agonist_ch2

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARg_BLA_Agonist_ch2 is an assay readout measuring reporter gene via receptor activity and designed using inducible reporter (beta lactamase induction) detected with GAL4 b-lactamase reporter gene.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.168054
Neutral control median absolute deviation, by plate:	6.24204
Positive control well median response value, by plate:	99.5724
Positive control well median absolute deviation, by plate:	14.524
Negative control well median, by plate:	1549.3087291266
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.37
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	16
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-592.31
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-36.12

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.58264421980395
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.4958653188237

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human HEK293T peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARg_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARg_BLA_Agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene PPARG. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_PPARg_BLA_Agonist_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_PPARg_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0933707
Neutral control median absolute deviation, by plate:	3.48718
Positive control well median response value, by plate:	99.8048
Positive control well median absolute deviation, by plate:	6.46131
Negative control well median, by plate:	441.1364058013
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.7
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	14
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	28.66
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-953.1
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-34.37

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.08360537976913
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

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Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0994903
Neutral control median absolute deviation, by plate:	6.23031
Positive control well median response value, by plate:	45.212
Positive control well median absolute deviation, by plate:	8.61949
Negative control well median, by plate:	841.2683396827
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.01
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	7.17
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-437.8
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-61.54

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.5073840720356
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.0443044322136

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.42003
Neutral control median absolute deviation, by plate:	23.2258
Positive control well median response value, by plate:	-100.508
Positive control well median absolute deviation, by plate:	12.6568
Negative control well median, by plate:	261.3667997393
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4.31
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	238.77
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-58.14

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	15.2972999678404
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	91.7837998070424

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_antagonist_ratio

Assay Title: Tox21/NCGC human HEK293 peroxisome proliferator-activated receptor gamma assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARg_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARg_BLA_antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene PPARG. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
Tox21_PPARg_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_PPARg_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.154416
Neutral control median absolute deviation, by plate:	10.0587
Positive control well median response value, by plate:	-100.233
Positive control well median absolute deviation, by plate:	5.50551
Negative control well median, by plate:	162.9282362548
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.53
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-9.95
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	583.55
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-61.24

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.9970277415496
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	35.9821664492976

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PPARg_BLA_antagonist_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_PPARg_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_PPARg_BLA_antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
Tox21_PPARg_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_PPARg_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_PPARg_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.209187
Neutral control median absolute deviation, by plate:	13.8117
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	109.5311992432
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	unspecified
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-62.94

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	9.1312358619699
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	54.7874151718194

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T progesterone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Agonist_viability

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

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References:

unspecified

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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4.3 Assay Documentation Source

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

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EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Antagonist_ratio

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T progesterone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Antagonist_viability

Assay Title: Tox21/NCGC human PR-UAS-bla-HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent PR-UAS-bla-HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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unspecified

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Agonist_ch1

Assay Title: TOX21_PR_BLA_Followup_Agonist_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Agonist_ch2

Assay Title: TOX21_PR_BLA_Followup_Agonist_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Agonist_ratio

Assay Title: TOX21_PR_BLA_Followup_Agonist_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Agonist_viability

Assay Title: TOX21_PR_BLA_Followup_Agonist_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Antagonist_ch1

Assay Title: TOX21_PR_BLA_Followup_Antagonist_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Antagonist_ch2

Assay Title: TOX21_PR_BLA_Followup_Antagonist_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Antagonist_ratio

Assay Title: TOX21_PR_BLA_Followup_Antagonist_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_BLA_Followup_Antagonist_viability

Assay Title: TOX21_PR_BLA_Followup_Antagonist_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_LUC_Followup_Agonist

Assay Title: TOX21_PR_LUC_Followup_Agonist

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

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unspecified

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_LUC_Followup_Agonist_viability

Assay Title: TOX21_PR_LUC_Followup_Agonist_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_LUC_Followup_Antagonist

Assay Title: TOX21_PR_LUC_Followup_Antagonist

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PR_LUC_Followup_Antagonist_viability

Assay Title: TOX21_PR_LUC_Followup_Antagonist_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_PXR_Agonist

Assay Title: TOX21_PXR_Agonist

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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3.2 Assay Performance

Assay Performance Measures:

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TOX21_PXR_viability

Assay Title: TOX21_PXR_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
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Positive control well median response value, by plate:	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RAR_LUC_Agonist

Assay Title: Tox21/NCGC mouse C3H10T1/2 retinoic acid receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent C3H10T1/2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.836468
Neutral control median absolute deviation, by plate:	1.96101
Positive control well median response value, by plate:	99.191
Positive control well median absolute deviation, by plate:	5.48435
Negative control well median, by plate:	196.148679673
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.77
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	50.85
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	105.07
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	2.07

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.83869932137985
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RAR_LUC_Agonist_viability

Assay Title: Tox21/NCGC mouse C3H10T1/2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent C3H10T1/2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.737514
Neutral control median absolute deviation, by plate:	2.87194
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	103.2984139787
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-1.47

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.53553439731881
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RAR_LUC_Antagonist

Assay Title: Tox21/NCGC mouse C3H10T1/2 retinoic acid receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent C3H10T1/2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.57111
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	2.31557
Negative control well median, by plate:	140.684670911
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.67
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-11.67
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.95899959044
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.75399754264

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RAR_LUC_Antagonist_viability

Assay Title: Tox21/NCGC mouse C3H10T1/2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent C3H10T1/2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0.395657
Neutral control median absolute deviation, by plate:	3.49768
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	101.8074463364
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	1.34

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.81181261800875
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RORg_LUC_CHO_Antagonist

Assay Title: Tox21/NCGC chinese hamster CHO-K1 RAR-related orphan receptor C assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent CHO-K1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-1.84907
Neutral control median absolute deviation, by plate:	11.9353
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.6148
Negative control well median, by plate:	105.5112679781
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.58
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	7.71
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-0.82

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of

xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	8.68420845631059
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	52.1052507378635

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RORg_LUC_CHO_Antagonist_viability

Assay Title: Tox21/NCGC chinese hamster CHO-K1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent CHO-K1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	-7.6058
Neutral control median absolute deviation, by plate:	7.15464
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	103.24596482915
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-0.89

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.0881472477345
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_FLO_00hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.8441
Positive control well median response value, by plate:	0.106813
Positive control well median absolute deviation, by plate:	5.07993
Negative control well median, by plate:	3807.707778039
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.02
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.74509318791379
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_FLO_08hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.03555
Positive control well median response value, by plate:	256.265
Positive control well median absolute deviation, by plate:	15.2003
Negative control well median, by plate:	4021.0794944423
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.74
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	36.23
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.92510785122456
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	23.5506471073474

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

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References:

unspecified

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_FLO_16hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.6927
Positive control well median response value, by plate:	228.265
Positive control well median absolute deviation, by plate:	10.5483
Negative control well median, by plate:	4534.3889496018
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.76
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	29.6
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.17946253309319
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.0767751985591

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_FLO_24hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.4067
Positive control well median response value, by plate:	241.623
Positive control well median absolute deviation, by plate:	15.9792
Negative control well median, by plate:	4007.947077885
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.69
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	29.17
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.47705368342379
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.8623221005427

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_FLO_32hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.76312
Positive control well median response value, by plate:	320.893
Positive control well median absolute deviation, by plate:	27.3743
Negative control well median, by plate:	3855.4763760461
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.66
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	37.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.73831980341741
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.4299188205045

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_FLO_40hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.76616
Positive control well median response value, by plate:	425.253
Positive control well median absolute deviation, by plate:	22.4678
Negative control well median, by plate:	4068.5493947172
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.78
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	18
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	48.28
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.75633195144065
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.5379917086439

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_GLO_00hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	10.6943
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	223.3685693967
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.2546809080829
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.5280854484974

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_GLO_08hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.45811
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	100.2151783176
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.04646609105207
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.2787965463124

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_GLO_16hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.47919
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	106.9580949244
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.0177086637968
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.1062519827808

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_GLO_24hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.86365
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	103.523512601
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.08919407864135
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.5351644718481

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_GLO_32hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.62798
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	104.0194353847
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.18706647811088
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	25.1223988686653

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEK293_GLO_40hr_viability

Assay Title: Tox21/NCGC human HEK293 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.57069
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	103.6581461342
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.15089564121323
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	24.9053738472794

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_FLO_00hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.10563
Positive control well median response value, by plate:	-6.29228
Positive control well median absolute deviation, by plate:	5.78753
Negative control well median, by plate:	4946.3491084572
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.79164116752995
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.7498470051797

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_FLO_08hr_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.80574
Positive control well median response value, by plate:	280.194
Positive control well median absolute deviation, by plate:	16.2137
Negative control well median, by plate:	6262.0118824559
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.73
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	33.21
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.68879869481702
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	28.1327921689021

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_FLO_16hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.94073
Positive control well median response value, by plate:	263.649
Positive control well median absolute deviation, by plate:	13.839
Negative control well median, by plate:	6457.6463557393
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.74
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	15
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	34.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.60782660753093
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.6469596451856

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

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References:

unspecified

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_FLO_24hr_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.61774
Positive control well median response value, by plate:	333.212
Positive control well median absolute deviation, by plate:	21.6999
Negative control well median, by plate:	5715.6329997676
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.72
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	42.63
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.57167345723585
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.4300407434151

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_FLO_32hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.87335
Positive control well median response value, by plate:	461.365
Positive control well median absolute deviation, by plate:	23.4551
Negative control well median, by plate:	5865.8678012207
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.79
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	17
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	58.53
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.88012714978376
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.2807628987026

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_FLO_40hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.25827
Positive control well median response value, by plate:	519.224
Positive control well median absolute deviation, by plate:	22.5312
Negative control well median, by plate:	5646.7651782225
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.82
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	71.09
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.48809608184861
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.9285764910917

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_GLO_00hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.27391
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	91.4111977132
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.23642796877449
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_GLO_08hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.41129
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	100.1071494222
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.79765049419046
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_GLO_16hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.67545
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.7789878932
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.42693580835246
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_GLO_24hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.24893
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	99.3546901405
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.22724010465665
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_GLO_32hr_ctrl_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.26975
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	104.6055915294
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.38797001670782
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RT_HEPG2_GLO_40hr_viability

Assay Title: Tox21/NCGC human HepG2 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HepG2 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.92294
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	100.7041204739
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	1
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.78454044898115
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RXR_BLA_Agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	6.77695
Positive control well median response value, by plate:	-11.5572
Positive control well median absolute deviation, by plate:	4.03658
Negative control well median, by plate:	57.2702992766
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.67
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-6.9
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	4.32

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.3524480628587
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.1146883771522

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RXR_BLA_Agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.50104
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	15.807
Negative control well median, by plate:	1058.2829602788
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.28
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	6
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	13.12
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	46.5
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	3.42

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.90395808901334
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.42374853408

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RXR_BLA_Agonist_ratio

Assay Title: Tox21/NCGC human HEK293T retinoid X receptor, alpha assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.08593
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	7.9299
Negative control well median, by plate:	333.038013596
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.64
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	11
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	24.33
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	63
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	2.43

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.27839049691729
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_RXR_BLA_Agonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.59894
Positive control well median response value, by plate:	-9.70694
Positive control well median absolute deviation, by plate:	7.22506
Negative control well median, by plate:	120.9308280825
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-1.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-4.61
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	4.29

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.43837580224682
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	26.6302548134809

Chemical Library Scope and Limitations:

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4.1 Resources

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References:

unspecified

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Agonist_ch1

Assay Title: TOX21_SBE_BLA_Agonist_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Agonist_ch2

Assay Title: TOX21_SBE_BLA_Agonist_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Agonist_ratio

Assay Title: TOX21_SBE_BLA_Agonist_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Agonist_viability

Assay Title: TOX21_SBE_BLA_Agonist_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

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References:

unspecified

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AOP, Adverse Outcome Pathway

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Antagonist_ch1

Assay Title: TOX21_SBE_BLA_Antagonist_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Antagonist_ch2

Assay Title: TOX21_SBE_BLA_Antagonist_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Antagonist_ratio

Assay Title: TOX21_SBE_BLA_Antagonist_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SBE_BLA_Antagonist_viability

Assay Title: TOX21_SBE_BLA_Antagonist_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SSH_3T3_GLI3_Agonist

Assay Title: Tox21/NCGC mouse NIH/3T3 GLI family zinc finger 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

adherent NIH/3T3 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	0.911272
Positive control well median response value, by plate:	-12.6442
Positive control well median absolute deviation, by plate:	0.516168
Negative control well median, by plate:	338.8044397792
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.68
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-15.5
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

GLI family zinc finger 3

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.59301128463255
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SSH_3T3_GLI3_Agonist_viability

Assay Title: Tox21/NCGC mouse NIH/3T3 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

adherent NIH/3T3 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	11.1444
Positive control well median response value, by plate:	-98.8388
Positive control well median absolute deviation, by plate:	2.82748
Negative control well median, by plate:	120.6213239665
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.6
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.9
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.74374658540332
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_SSH_3T3_GLI3_Antagonist

Assay Title: Tox21/NCGC mouse NIH/3T3 GLI family zinc finger 3 assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

adherent NIH/3T3 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	11.8873
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	1.04308
Negative control well median, by plate:	131.830097671
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.61
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.41
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

GLI family zinc finger 3

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.36703975915985
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	44.2022385549591

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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TOX21_SSH_3T3_GLI3_Antagonist_viability

Assay Title: Tox21/NCGC mouse NIH/3T3 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

adherent NIH/3T3 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.69272
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	0.617363
Negative control well median, by plate:	111.4188280231
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.84
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-21
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-21.31
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.48726544475101
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_COA_Antagonist_Followup_ch1

Assay Title: TOX21_TR_COA_Antagonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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unspecified

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_COA_Antagonist_Followup_ch2

Assay Title: TOX21_TR_COA_Antagonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_COA_Antagonist_Followup_ratio

Assay Title: TOX21_TR_COA_Antagonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_LUC_GH3_Agonist

Assay Title: Tox21/NCGC rat GH3 thyroid hormone receptor, alpha|thyroid hormone receptor, beta|thyroid hormone receptor beta|thyroid hormone receptor, alpha | thyroid hormone receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_TR_LUC_GH3_Agonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_TR_LUC_GH3_Agonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, gain-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene THRA and THRB. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent GH3 cell line

Basic Procedure:
Tox21_TR_LUC_GH3_Agonist, is one of 1 assay component(s) measured or calculated from the Tox21_TR_LUC_GH3_Agonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TR_LUC_GH3_Agonist is a cell-based, single-readout assay that uses GH3, a rat pituitary gland cell line, with measurements taken at 28 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.0399883
Neutral control median absolute deviation, by plate:	1.60235
Positive control well median response value, by plate:	99.5925
Positive control well median absolute deviation, by plate:	12.6427
Negative control well median, by plate:	129.3037327269
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.57
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	62.07
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-2484.75
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-39.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA|NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	0.642764372838
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Freitas J, Miller N, Mengeling BJ, Xia M, Huang R, Houck K, Rietjens IM, Furlow JD, Murk AJ. Identification of thyroid hormone receptor active compounds using a quantitative high-throughput screening platform. Curr Chem Genomics Transl Med. 2014 Mar 7;8:36-46. doi: 10.2174/2213988501408010036. eCollection 2014. PubMed PMID: 24772387; PubMed Central PMCID: PMC3999704.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_LUC_GH3_Agonist_Followup

Assay Title: TOX21_TR_LUC_GH3_Agonist_Followup

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_LUC_GH3_Antagonist

Assay Title: Tox21/NCGC rat GH3 thyroid hormone receptor, alpha|thyroid hormone receptor, beta|thyroid hormone receptor beta|thyroid hormone receptor, alpha | thyroid hormone receptor, beta assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_TR_LUC_GH3_Antagonist was analyzed into 1 assay endpoint. This assay endpoint, Tox21_TR_LUC_GH3_Antagonist, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, loss-of-signal activity can be used to understand changes in the reporter gene as they relate to the gene THRA and THRB. Furthermore, this assay endpoint can be referred to as a primary readout, because the performed assay has only produced 1 assay endpoint. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the nuclear receptor intended target family, where the subfamily is non-steroidal.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent GH3 cell line

Basic Procedure:
Tox21_TR_LUC_GH3_Antagonist, is one of 1 assay component(s) measured or calculated from the Tox21_TR_LUC_GH3_Antagonist assay. It is designed to make measurements of luciferase induction, a form of inducible reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TR_LUC_GH3_Antagonist is a cell-based, single-readout assay that uses GH3, a rat pituitary gland cell line, with measurements taken at 28 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.128026
Neutral control median absolute deviation, by plate:	7.91022
Positive control well median response value, by plate:	-100.098
Positive control well median absolute deviation, by plate:	2.79063
Negative control well median, by plate:	217.1818723912
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.68
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-12.63
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	774.67
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-60.42

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA|NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.73945711206963
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	22.4367426724178

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich

chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Freitas J, Miller N, Mengeling BJ, Xia M, Huang R, Houck K, Rietjens IM, Furlow JD, Murk AJ. Identification of thyroid hormone receptor active compounds using a quantitative high-throughput screening platform. Curr Chem Genomics Transl Med. 2014 Mar 7;8:36-46. doi: 10.2174/2213988501408010036. eCollection 2014. PubMed PMID: 24772387; PubMed Central PMCID: PMC3999704.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_LUC_GH3_Antagonist_Followup

Assay Title: TOX21_TR_LUC_GH3_Antagonist_Followup

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_LUC_GH3_Antagonist_viability

Assay Title: Tox21/NCGC rat GH3 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Tox21_TR_LUC_GH3_Antagonist_viability

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent GH3 cell line

Basic Procedure:
Tox21_TR_LUC_GH3_Antagonist_viability is an assay readout measuring viability via receptor activity and designed using NA (NA) detected with Luciferase-coupled ATP quantitation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TR_LUC_GH3_Antagonist is a cell-based, single-readout assay that uses GH3, a rat pituitary gland cell line, with measurements taken at 28 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.119763
Neutral control median absolute deviation, by plate:	9.09814
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	154.3454190063
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-75.57

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is

considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	6.67922423139783
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	40.075345388387

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Freitas J, Miller N, Mengeling BJ, Xia M, Huang R, Houck K, Rietjens IM, Furlow JD, Murk AJ. Identification of thyroid hormone receptor active compounds using a quantitative high-throughput screening platform. Curr Chem Genomics Transl Med. 2014 Mar 7;8:36-46. doi: 10.2174/2213988501408010036. eCollection 2014. PubMed PMID: 24772387; PubMed Central PMCID: PMC3999704.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Agonist_Followup_ch1

Assay Title: TOX21_TR_RXR_BLA_Agonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Agonist_Followup_ch2

Assay Title: TOX21_TR_RXR_BLA_Agonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Agonist_Followup_ratio

Assay Title: TOX21_TR_RXR_BLA_Agonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Agonist_Followup_viability

Assay Title: TOX21_TR_RXR_BLA_Agonist_Followup_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Antagonist_Followup_ch1

Assay Title: TOX21_TR_RXR_BLA_Antagonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Antagonist_Followup_ch2

Assay Title: TOX21_TR_RXR_BLA_Antagonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Antagonist_Followup_ratio

Assay Title: TOX21_TR_RXR_BLA_Antagonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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References:

unspecified

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TR_RXR_BLA_Antagonist_Followup_viability

Assay Title: TOX21_TR_RXR_BLA_Antagonist_Followup_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRA_COA_Agonist_Followup_ch1

Assay Title: TOX21_TRA_COA_Agonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRA_COA_Agonist_Followup_ch2

Assay Title: TOX21_TRA_COA_Agonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

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References:

unspecified

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRA_COA_Agonist_Followup_ratio

Assay Title: TOX21_TRA_COA_Agonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRA_COA_Antagonist_Followup_ch1

Assay Title: TOX21_TRA_COA_Antagonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRA_COA_Antagonist_Followup_ch2

Assay Title: TOX21_TRA_COA_Antagonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRA_COA_Antagonist_Followup_ratio

Assay Title: TOX21_TRA_COA_Antagonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Agonist_Followup_ch1

Assay Title: TOX21_TRB_BLA_Agonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

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Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Agonist_Followup_ch2

Assay Title: TOX21_TRB_BLA_Agonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Agonist_Followup_ratio

Assay Title: TOX21_TRB_BLA_Agonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Agonist_Followup_viability

Assay Title: TOX21_TRB_BLA_Agonist_Followup_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Antagonist_Followup_ch1

Assay Title: TOX21_TRB_BLA_Antagonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Antagonist_Followup_ch2

Assay Title: TOX21_TRB_BLA_Antagonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Antagonist_Followup_ratio

Assay Title: TOX21_TRB_BLA_Antagonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_BLA_Antagonist_Followup_viability

Assay Title: TOX21_TRB_BLA_Antagonist_Followup_viability

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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References:

unspecified

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CV, Coefficient of Variation

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EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_COA_Agonist_Followup_ch1

Assay Title: TOX21_TRB_COA_Agonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
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Negative control well median absolute deviation value, by plate:	unspecified
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SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
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Assay Summary:

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Assay Throughput:

1536 well

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Basic Procedure:
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Proprietary Elements:

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Caveats:

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1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_COA_Agonist_Followup_ratio

Assay Title: TOX21_TRB_COA_Agonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_COA_Antagonist_Followup_ch1

Assay Title: TOX21_TRB_COA_Antagonist_Followup_ch1

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_COA_Antagonist_Followup_ch2

Assay Title: TOX21_TRB_COA_Antagonist_Followup_ch2

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

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unspecified

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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SULT, Sulfotransferases

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Date of Assay Document Creation:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRB_COA_Antagonist_Followup_ratio

Assay Title: TOX21_TRB_COA_Antagonist_Followup_ratio

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
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Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRHR_HEK293_Agonist

Assay Title: TOX21_TRHR_HEK293_Agonist

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TRHR_HEK293_Antagonist

Assay Title: TOX21_TRHR_HEK293_Antagonist

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536 well

Experimental System:

unspecified

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_Agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	8.2985
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	1.52256
Negative control well median, by plate:	125.4722348378
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.7
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	12
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	12.05
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.27126094389937
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	31.6275656633962

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_Agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.62073
Positive control well median response value, by plate:	8.31303
Positive control well median absolute deviation, by plate:	6.16208
Negative control well median, by plate:	282.6882084639
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	1.8
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.35265748281681
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20.1159448969009

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_Agonist_ratio

Assay Title: Tox21/NCGC human HEK293T thyroid stimulating hormone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_TSHR_Agonist_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_TSHR_Agonistt assay. It is designed to make measurements of TSHR agonism, with cAMP as an indicator of TSHR activation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	7.7611
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	1.44387
Negative control well median, by plate:	105.2561726902
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.72
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	13
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	12.88
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid stimulating hormone receptor

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.64129367543786
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.8477620526272

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal

toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	3.05575
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	12.7448
Negative control well median, by plate:	4780.8628962128
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.52
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-32.73
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.90228676304723
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

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Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.68424
Positive control well median response value, by plate:	-3.31756
Positive control well median absolute deviation, by plate:	4.74916
Negative control well median, by plate:	103.505702475
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-0.7
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.2181984949422
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

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References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Date of Assay Document Creation:

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Author of Revisions:

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_Antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T thyroid stimulating hormone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_TSHR_Antagonist_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_TSHR_Antagonist assay. It is designed to make measurements of TSHR antagonism, with cAMP as an indicator of TSHR activation.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	2.80088
Positive control well median response value, by plate:	-100
Positive control well median absolute deviation, by plate:	11.724
Negative control well median, by plate:	1413.0740099752
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.56
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-8
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-35.7
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid stimulating hormone receptor

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	1.7350029827067
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

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toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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Assay Source Information:

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CV, Coefficient of Variation

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DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_wt_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_wt is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.49155
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	1.13588
Negative control well median, by plate:	2632.5748289911
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.83
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	22
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	22.26
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.12071491051026
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_wt_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_wt is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.30388
Positive control well median response value, by plate:	9.61958
Positive control well median absolute deviation, by plate:	4.97189
Negative control well median, by plate:	297.026691244
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	1
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	2.22
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	2.77206321796152
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_TSHR_wt_ratio

Assay Title: Tox21/NCGC human HEK293T thyroid stimulating hormone receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_TSHR_wt_ratio, is one of 1 assay component(s) measured or calculated from the Tox21_TSHR_wt assay. It is designed to indicate background cAMP activity.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_TSHR_wt is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 0.5 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	0
Neutral control median absolute deviation, by plate:	4.1604
Positive control well median response value, by plate:	100
Positive control well median absolute deviation, by plate:	1.13238
Negative control well median, by plate:	102.2717479541
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.84
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	23
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	24.04
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	Inf
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	Inf

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

thyroid stimulating hormone receptor

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss

function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	89.999999999999 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.28165373446722
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	20

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction

modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_agonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.122072
Neutral control median absolute deviation, by plate:	7.50854
Positive control well median response value, by plate:	-16.0466
Positive control well median absolute deviation, by plate:	3.43393
Negative control well median, by plate:	51.4109542662
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-2.08
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	118.71
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-62.55

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.98272374985238
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	29.8963424991143

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_agonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.168503
Neutral control median absolute deviation, by plate:	8.68585
Positive control well median response value, by plate:	98.4703
Positive control well median absolute deviation, by plate:	24.0966
Negative control well median, by plate:	2064.6702375442
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	11.4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-571.71
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-49.98

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	5.5343508329109
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	33.2061049974654

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_agonist_ratio

Assay Title: Tox21/NCGC human HEK293T cytochrome P450, family 24, subfamily A, polypeptide 1|vitamin D (1,25- dihydroxyvitamin D3) receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_VDR_BLA_Agonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_VDR_BLA_agonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene CYP24A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cyp intended target family, where the subfamily is xenobiotic metabolism.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_VDR_BLA_Agonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_VDR_BLA_Agonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.127676
Neutral control median absolute deviation, by plate:	6.0542
Positive control well median response value, by plate:	99.242
Positive control well median absolute deviation, by plate:	13.0312
Negative control well median, by plate:	424.885326369
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.42
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	16.45
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-698.9
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-42.35

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	3.52202670091944
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	21.1321602055166

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_Agonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_VDR_BLA_Agonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_VDR_BLA_Agonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_VDR_BLA_Agonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_VDR_BLA_Agonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Agonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.15416
Neutral control median absolute deviation, by plate:	8.27586
Positive control well median response value, by plate:	1.26533
Positive control well median absolute deviation, by plate:	7.99483
Negative control well median, by plate:	110.6789996725
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	0
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	0.18
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-6.38
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-51.8

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.53166577259249
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.1899946355549

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial

availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_Antagonist_ch1

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.124661
Neutral control median absolute deviation, by plate:	6.17465
Positive control well median response value, by plate:	29.1004
Positive control well median absolute deviation, by plate:	14.0458
Negative control well median, by plate:	1081.2966156852
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	2
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	4.71
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	-244.45
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-49.5

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	4.64972192151852
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	27.8983315291111

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

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Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

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EDC, Endocrine disrupting chemicals

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Contact Information:

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Date of Assay Document Creation:

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EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_Antagonist_ch2

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.580121
Neutral control median absolute deviation, by plate:	24.8619
Positive control well median response value, by plate:	-100.108
Positive control well median absolute deviation, by plate:	5.25496
Negative control well median, by plate:	169.9587423067
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	0.07
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-4
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-4
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	172.5
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-44.8

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the

following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	11.9999191832974
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	71.9995150997844

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_antagonist_ratio

Assay Title: Tox21/NCGC human HEK293T cytochrome P450, family 24, subfamily A, polypeptide 1|vitamin D (1,25- dihydroxyvitamin D3) receptor assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_VDR_BLA_Antagonist_ratio was analyzed into 1 assay endpoint. This assay endpoint, Tox21_VDR_BLA_antagonist_ratio, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of inducible reporter, measures of receptor for gain-of-signal activity can be used to understand the reporter gene at the pathway-level as they relate to the gene CYP24A1. Furthermore, this assay endpoint can be referred to as a primary readout, because this assay has produced multiple assay endpoints where this one serves a reporter gene function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cyp intended target family, where the subfamily is xenobiotic metabolism.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_VDR_BLA_Antagonist_ratio, is one of 2 assay component(s) measured or calculated from the Tox21_VDR_BLA_Antagonist assay. It is designed to make measurements of beta lactamase induction, a form of inducible reporter, as detected with fluorescence intensity signals by GAL4 b-lactamase reporter gene technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.227618
Neutral control median absolute deviation, by plate:	12.7304
Positive control well median response value, by plate:	-100.086
Positive control well median absolute deviation, by plate:	4.6108
Negative control well median, by plate:	152.1552500965
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.48
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-7
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-7.83
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	424.66
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-53.06

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

NA|NA

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.44565572024603
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	44.6739343214762

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

TOX21_VDR_BLA_antagonist_viability

Assay Title: Tox21/NCGC human HEK293T unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

Data from the assay component Tox21_VDR_BLA_Antagonist_viability was analyzed into 1 assay endpoint. This assay endpoint, Tox21_VDR_BLA_antagonist_viability, was analyzed in the positive fitting direction relative to DMSO as the negative control and baseline of activity. Using a type of viability reporter, measures of ATP for loss-of-signal activity can be used to understand the viability at the cellular-level. Furthermore, this assay endpoint can be referred to as a secondary readout, because this assay has produced multiple assay endpoints where this one serves a viability function. To generalize the intended target to other relatable targets, this assay endpoint is annotated to the cell cycle intended target family, where the subfamily is cytotoxicity.

1.2 Assay Definition

Assay Throughput:

1536-well plate

Experimental System:

adherent HEK293T cell line

Basic Procedure:
Tox21_VDR_BLA_Antagonist_viability, is one of 2 assay component(s) measured or calculated from the Tox21_VDR_BLA_Antagonist assay. It is designed to make measurements of atp content, a form of viability reporter, as detected with bioluminescence signals by Luciferase-coupled ATP quantitation technology.

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

Tox21_VDR_BLA_Antagonist is a cell-based, single-readout assay that uses HEK293T, a human kidney cell line, with measurements taken at 24 hours after chemical dosing in a 1536-well plate.

Assay Quality Statistics:

Neutral control well median response value, by plate:	-0.225107
Neutral control median absolute deviation, by plate:	11.073
Positive control well median response value, by plate:	-100.01
Positive control well median absolute deviation, by plate:	0.120802
Negative control well median, by plate:	116.8805161824
Negative control well median absolute deviation value, by plate:	unspecified
Z’ (median across all plates, using positive control wells):	0.66
Z’ (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	-9
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	-8.93
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	607.57
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	-50.52

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	15
Target (nominal) number of replicates:	3
Standard minimum concentration tested:	0.001 µM
Standard maximum concentration tested:	79.9999999999993 µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	7.52699616165189
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	45.1619769699113

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives,

toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

Tox21 is an interagency agreement between the NIH, NTP, FDA and EPA. NIH Chemical Genomics Center (NCGC) is the primary screening facility running ultra high throughput screening assays across a large interagency-developed chemical library.

References:

Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011 Aug;119(8):1142-8. doi: 10.1289/ehp.1002952. Epub 2011 May 4. PubMed PMID: 21543282; PubMed Central PMCID: PMC3237348.

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_CellCount_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_K2_ADFPvLipid_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_K2_ADFPvLipid_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_LipidDropletDiam_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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4.3 Assay Documentation Source

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_LipidDropletDiam_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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Vala_A1_LipidPxlInt_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_LipidPxlInt_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_PCC_ADFPvLipid_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_PCC_ADFPvLipid_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_PCC_PerilipinvLipid_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

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References:

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_PCC_PerilipinvLipid_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_PctDifferenCells_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A1_PctDifferenCells_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_CellCount_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_K1_ADFPvLipid_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_K1_ADFPvLipid_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_K2_LipidvADFP_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_K2_LipidvADFP_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_LipidDropletDiam_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Vala_A2_LipidDropletDiam_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_LipidPxlInt_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_LipidPxlInt_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_PCC_LipidvPerilipin_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_PCC_LipidvPerilipin_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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FBS, Fetal Bovine Serum

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MIE, Molecular Initiating Event

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NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_A2_PctDifferenCells_dn

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Vala_A2_PctDifferenCells_up

Assay Title: VALA Sciences human subcutaneous preadipocyte unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous preadipocyte primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
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Z' (median across all plates, using negative control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_BC1_CellCount_dn

Assay Title: VALA Sciences human microvascular endothelial and neonatal dermis unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent microvascular endothelial and neonatal dermis primary cell co-culture

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

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Vala_BC1_PCC_betacateninvNucleus_dn

Assay Title: VALA Sciences human microvascular endothelial and neonatal dermis unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent microvascular endothelial and neonatal dermis primary cell co-culture

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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384-well plate

Experimental System:

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Basic Procedure:
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Proprietary Elements:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

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Assay Quality Statistics:

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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

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Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above

the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation); if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_ECAD_CellCount_dn

Assay Title: VALA Sciences human A431 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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SSMD, Strictly Standardized Mean Difference

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_ECAD_ECADCellInt_dn

Assay Title: VALA Sciences human A431 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_ECAD_ECADCellInt_up

Assay Title: VALA Sciences human A431 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_ECAD_ECADMembraneInt_dn

Assay Title: VALA Sciences human A431 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

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References:

unspecified

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SULT, Sulfotransferases

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1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_ECAD_TSA_dn

Assay Title: VALA Sciences human A431 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_ECAD_TSA_up

Assay Title: VALA Sciences human A431 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent A431 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_CellCount_dn

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_LipidDropletDiam_dn

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_LipidDropletDiam_up

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_PCC_pHSLvLipid_dn

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_PCC_pHSLvLipid_up

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_PCC_pPerilipinvLipid_dn

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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EDC, Endocrine disrupting chemicals

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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Vala_L2_PCC_pPerilipinvLipid_up

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_PctDifferenCells_dn

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_L2_PctDifferenCells_up

Assay Title: VALA Sciences human subcutaneous fat cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent subcutaneous fat cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_CellCount_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_LipidDropletArea_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_LipidDropletArea_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Vala_LD1_LipidDropletDiam_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

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1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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Vala_LD1_LipidDropletDiam_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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Vala_LD1_LipidPxlInt_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
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Z' (median across all plates, using positive control wells):	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
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Standard minimum concentration tested:	unspecified µM
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References:

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Assay Source Information:

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Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

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Biological Response:

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Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_M2_ADFPvLipid_m_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_M2_ADFPvLipid_m_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_M2_ADFPvLipid_md_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD1_M2_ADFPvLipid_md_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_CellCount_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_LipidDropletDiam_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_LipidDropletDiam_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_LipidPxlInt_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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SULT, Sulfotransferases

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4.3 Assay Documentation Source

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Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_LipidPxlInt_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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SULT, Sulfotransferases

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Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_M2_ADFPvLipid_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_M2_ADFPvLipid_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_PCC_ADFPvlipid_dn

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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Date of Assay Document Creation:

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5. Potential Regulatory Applications

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_LD2_PCC_ADFPvlipid_up

Assay Title: VALA Sciences human HuH7 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent HuH7 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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Assay Source Information:

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References:

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_CellCount_dn

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_Flk1Int_dn

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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ATCC, American Tissue Culture Collection

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NCI, Normalized Cell Index

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Author of Revisions:

EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_Flk1Int_up

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_MOC_NucleusFoxA2_dn

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_MOC_NucleusFoxA2_up

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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919-541-4219

Date of Assay Document Creation:

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EPA NCCT

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Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_PCC_NucleusvFlk1_dn

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_PCC_NucleusvFlk1_up

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_PCC_NucleusvFoxA2_dn

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_MEF1_PCC_NucleusvFoxA2_up

Assay Title: VALA Sciences mouse embryonic stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent embryonic stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_NO1_CellCount_dn

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_NO1_M1_PrePostSynapticImage_dn

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

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Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_NO1_M1_PrePostSynapticImage_up

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_NO1_M2_PrePostNeuriteImage_dn

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_NO1_M2_PrePostNeuriteImage_up

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

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Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

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Vala_NO1_NeuriteArea_dn

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_NO1_NeuriteArea_up

Assay Title: VALA Sciences human neuronal stem cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent neuronal stem cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_PBCD_CellCount_dn

Assay Title: VALA Sciences human T6PNE unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T6PNE cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_PBCD_FractionCellsGFPabvbgd_dn

Assay Title: VALA Sciences human T6PNE unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T6PNE cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

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Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_PBCD_FractionCellsGFPabvbgd_up

Assay Title: VALA Sciences human T6PNE unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T6PNE cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

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EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

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Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_PBCD_GFPNucleusInt_dn

Assay Title: VALA Sciences human T6PNE unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T6PNE cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

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Vala_PBCD_GFPNucleusInt_up

Assay Title: VALA Sciences human T6PNE unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent T6PNE cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

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919-541-4219

Date of Assay Document Creation:

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Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_SCP1_CellCount_dn

Assay Title: VALA Sciences human Ntera2 cl.D1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent Ntera2 cl.D1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

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CYP, Cytochrome P450s

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EDC, Endocrine disrupting chemicals

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Vala_SCP1_PCC_Oct3vNucleus_dn

Assay Title: VALA Sciences human Ntera2 cl.D1 unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent Ntera2 cl.D1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

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Assay Title: VALA Sciences human Ntera2 cl.D1 unspecified assay

1. Assay Descriptions

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unspecified

1.2 Assay Definition

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adherent Ntera2 cl.D1 cell line

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

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Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
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SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
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CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_VECAD_CellCount_dn

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

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919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_VECAD_TSA_dn

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_VECAD_TSA_up

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_VECAD_TSAMiMm_dn

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

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FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

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RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

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919-541-4219

Date of Assay Document Creation:

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

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Vala_VECAD_TSAMiMm_up

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

if the modeled top of the curve was above the established response cutoff; and if the Hill or Gain-Loss model had a lower AIC value than the Constant model. An AC50 (concentration in µM at 50% of maximum activity; modl_ga), Hill-slope (modl_gw for Hill, modl_gw (gain) and modl_lw (loss) for Gain-Loss functions), and maximum activity (modl_tp) were determined for each active test chemical. Winning model probability (modl_prob) and RMSE (modl_rmse) are also generated for each active chemical response series and all data are publicly available on the ToxCast data download page (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

The ToxCast chemical library was designed to capture a large spectrum of structurally and physicochemically diverse compounds. EPA's ToxCast inventory incorporates toxicity data-rich chemicals, chemicals spanning major use-categories, and chemicals with exposure potential, including but not limited to pesticides, antimicrobials, fragrances, green chemistry alternatives, food additives, toxicity reference compounds and failed pharmaceuticals. In addition to environmental or exposure concerns, chemical selection criteria also considered practical constraints, such as commercial availability, dimethyl sulfoxide (DMSO) solubility and stability, and suitability for testing in automated or semi-automated systems (e.g., low volatility and moderate LogP values). Operating within these constraints, there were three major, interrelated drivers for chemical selection: availability of animal toxicity data or mechanistic knowledge, exposure potential, and EPA regulatory interest. The first driver would provide the necessary in vivo and mechanistic data to anchor and validate subsequent prediction modeling efforts, whereas the latter two were intended to provide coverage of the chemical landscape to which humans and ecosystems are potentially exposed and for which toxicity data are mostly lacking.

4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

AIC, Akaike Information Criterion

AOP, Adverse Outcome Pathway

ATCC, American Tissue Culture Collection

CV, Coefficient of Variation

CYP, Cytochrome P450s

DMSO, Dimethyl Sulfoxide

EDC, Endocrine disrupting chemicals

ER, Estrogen Receptor

FBS, Fetal Bovine Serum

GST, Glutathione S-Transferase

MIE, Molecular Initiating Event

NCI, Normalized Cell Index

NR, Nuclear Receptor

RTCA, Real-Time Cell Analysis

SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

UGT, UDP-glucuronosyltransferase

4.3 Assay Documentation Source

Contact Information:

U.S. EPA National Center for Computational Toxicology (NCCT)

109 T.W. Alexander Drive (MD-B-205-1)
Research Triangle Park, NC 27711
919-541-4219

Date of Assay Document Creation:

25 September 2018

Author of Revisions:

EPA NCCT

5. Potential Regulatory Applications

Examples of end use scenarios could include, but are not limited to:

Support Category Formation and Read-Across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;

Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_VECAD_WholeCellInt_dn

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
Positive control well median absolute deviation, by plate:	unspecified
Negative control well median, by plate:	unspecified
Negative control well median absolute deviation value, by plate:	unspecified
Z' (median across all plates, using positive control wells):	unspecified
Z' (median across all plates, using negative control wells):	unspecified
SSMD (median across all plates, using positive control wells):	unspecified
SSMD (median across all plates, using negative control wells):	unspecified
Signal-to-noise (median across all plates, using positive control wells):	unspecified
Signal-to-noise (median across all plates, using negative control wells):	unspecified
Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

Nominal number of tested concentrations:	unspecified
Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
Standard maximum concentration tested:	unspecified µM
Baseline median absolute deviation for the assay – based on the response values at the 2 lowest tested concentrations (bmad):	unspecified
Response cutoff used to derive hit calls (e.g., 5*bmad, 10*bmad):	unspecified

Chemical Library Scope and Limitations:

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4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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CYP, Cytochrome P450s

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NCI, Normalized Cell Index

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SSMD, Strictly Standardized Mean Difference

SULT, Sulfotransferases

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Date of Assay Document Creation:

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EPA NCCT

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Priority Setting: The assay might help prioritize substances within an inventory for more detailed evaluation

Screening Level Assessment of a Biomarker or Mechanistic Activity or Response: The screening level assessment may be sufficient to identify a hazard provide a gauge of potency

Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA.

Vala_VECAD_WholeCellInt_up

Assay Title: VALA Sciences human vascular endothelial cell unspecified assay

1. Assay Descriptions

1.1 Overview

Assay Summary:

unspecified

1.2 Assay Definition

Assay Throughput:

384-well plate

Experimental System:

adherent vascular endothelial cell primary cell

Basic Procedure:
unspecified

Proprietary Elements:

Assay is non-proprietary.

Caveats:

The assay described here is intended to provide initial (screening) information about the capacity for a chemical to promote a biological response, and is intended to provide information on a large number of diverse compounds; caution is advised with extrapolation of these results to prediction of organism-level responses. The potential for a chemical to elicit adverse health outcomes in living systems is a function of multiple factors, and this assay is not intended to provide predictive details regarding long term or indirect adverse effects in complex biological systems, but can aid in the prioritization of compound selection for more resource intensive toxicity studies.

Status:

The assay is fully developed, and data are publicly available.

1.3 Assay References

Assay Source Information:

unspecified

Method Updates / Confirmatory Studies:

None reported.

2. Assay Component Descriptions

Assay Objectives:

unspecified

Assay Quality Statistics:

Neutral control well median response value, by plate:	unspecified
Neutral control median absolute deviation, by plate:	unspecified
Positive control well median response value, by plate:	unspecified
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Signal-to-noise (median across all plates, using positive control wells):	unspecified
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Signal-to-background (median across all plates, using positive control wells):	unspecified
Signal-to-background (median across all plates, using negative control wells):	unspecified
CV (median across all plates):	unspecified

3. Assay Endpoint Descriptions

3.1 Data Interpretation

Biological Response:

unspecified

Analytical Elements:

All statistical analyses were conducted using R programming language, employing tcpl package to generate model parameters and confidence intervals. Each chemical concentration series is fitted to three predictive models; a constant function (no activity), a 4-parameter Hill function and a gain-loss function (two sequential Hill functions, which allows for curves with both increasing and decreasing trajectories). The model which produces the lowest Akaike Information Criterion (AIC) value is considered the winning model and used in further analysis as the most appropriate predictor of xenobiotic effects. Biologically relevant response was determined based on a chemical fulfilling the following criteria; either the median of normalized response values at a single concentration falls above the signal noise band (in this assay, any response over 5 times the baseline median absolute deviation);

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Related ToxCast Assays:

3.2 Assay Performance

Assay Performance Measures:

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Target (nominal) number of replicates:	unspecified
Standard minimum concentration tested:	unspecified µM
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Chemical Library Scope and Limitations:

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4. Assay Documentation

4.1 Resources

Assay Source Information:

unspecified

References:

unspecified

4.2 Abbreviations and Definitions

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MIE, Molecular Initiating Event

NCI, Normalized Cell Index

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RTCA, Real-Time Cell Analysis

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4.3 Assay Documentation Source

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Date of Assay Document Creation:

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